

UNITED STATES PATENT AND TRADEMARK OFFICE

BEFORE THE PATENT TRIAL AND APPEAL BOARD

LCY Biotechnology Holding, Inc.,
Petitioner

v.

Radici Chimica S.p.A.,
Patent Owner

Patent No. 11,781,148

Issue Date: October 10, 2023

Title: BIOLOGICAL METHODS FOR PREPARING TERPENES

Case No. PGR2024-00044

**DECLARATION OF DR. HAL ALPER IN SUPPORT OF PETITIONER'S
REQUEST FOR POST-GRANT REVIEW**

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APPENDIX OF EXHIBITS

No.	Description	Referred To Herein As	Corresponding Hall-Ellis Exhibit No.
1001	U.S. Patent No. 11,781,148 (the “’148 Patent”)	’148 Patent	N/A
1002	’148 Patent Prosecution History	’148 PH	N/A
1003	PCT Publication No. WO2008042338 (“Bailey”) was filed by Microbia, Inc. on September 28, 2007, and published on April 10, 2008.	Bailey	N/A
1004	US20040265980 (“Zhang”) was filed by Cognis Corp. on July 29, 2003, and published on December 30, 2004.	Zhang	N/A
1005	Declaration of Dr. Hal S. Alper	Alper	N/A
1006	Declaration of Dr. Sylvia Hall-Ellis	Hall-Ellis Decl.	N/A
1007	ROBERT F. WEAVER, <i>Chapter 2: The Molecular Nature of Genes</i> , MOLECULAR BIOLOGY (McGraw Hill 5 th Ed. 2012).	Weaver 2012	N/A
1008	BRUCE ALBERTS ET AL., MOLECULAR BIOLOGY OF THE CELL, Garland Science (5 th Ed. 2008).	Alberts 2008	N/A
1009	Thomas J. Maimone & Phil S. Baran, <i>Modern Synthetic Efforts Toward Biologically Active Terpenes</i> , 3 N. CHEM. BIO. 7, 396-407 (2007).	Maimone 2007	N/A
1010	Sam Zwenger & Chhandak Basu, <i>Plant Terpenoids: Applications and Future Potentials</i> , 3 BIOTECHNOL. MOL. BIOL. REV. 1, 1-7 (2008).	Zwenger 2008	N/A
1011	John Blazeck & Hal S. Alper, <i>Promoter engineering: Recent Advances In Controlling Transcription At The Most Fundamental Level</i> , 8 BIOTECHNOL. J., 46-58 (2013).	Blazeck 2013	N/A

No.	Description	Referred To Herein As	Corresponding Hall-Ellis Exhibit No.
1012	Vikki M. Weake & Jerry L. Workman, <i>Inducible Gene Expression: Diverse Regulatory Mechanisms</i> , 11 NATURE REV. GENET., 426-437 (2010).	Weake 2010	N/A
1013	Katrin Weinhandl et al., <i>Carbon Source Dependent Promoters in Yeasts</i> , 13 MICROBIAL CELL FACTORIES 5, 1-17 (2014).	Weinhandl 2014	N/A
1014	Tamotsu Kanai et al., <i>An n-Alkane-Responsive Promoter Element Found in the Gene Encoding the Peroxisomal Protein of Candida tropicalis Does Not Contain a C₆ Zinc Cluster DNA-Binding Motif</i> , 182 J. BACTERIOL. 9, 2492-2497 (2000).	Kanai 2000	N/A
1015	Tamotsu Kanai et al., <i>An n-Alkane-Responsive Promoter Element Found in the Gene Encoding the Peroxisomal Protein of Candida tropicalis Does Not Contain a C₆ Zinc Cluster DNA-Binding Motif</i> , 182 J. BACTERIOL. 9, Figure 3 at 2496 (2000).	Kanai Fig. 3	N/A
1016	Yutaka Masuda et al., <i>Cloning and Characterization of the POX2 Gene in Candida maltosa</i> , 167 GENE 1-2, 157-161 (1995).	Masuda 1995	N/A
1017	Yutaka Masuda et al., <i>Cloning and Characterization of the POX2 Gene in Candida maltosa</i> , 167 GENE 1-2, Figure 2 at 159 (1995).	Masuda Fig. 2	N/A

No.	Description	Referred To Herein As	Corresponding Hall-Ellis Exhibit No.
1018	Michael E. Lee et al., <i>A Highly Characterized Yeast Toolkit for Modular, Multipart Assembly</i> , 4 ACS. SYNTH. BIOL. 9, 975-986 (2015).	Lee 2015	N/A
1019	J.P. van der Walt & J.A. von Arx, <i>The Yeast Genus Yarrowia gen. nov.</i> , 46 ANTONIE VAN LEEUWENHOEK, 517-521 (1980).	van der Walt 1980	N/A
1020	J.P. van der Walt et al., <i>Arxula gen. nov. (Candidaceae), a new anamorphic, arthroconidial yeast genus</i> , 57 ANTONIE VAN LEEUWENHOEK, 59-61 (1990).	van der Walt 1990	N/A
1021	David L. Craft et al., <i>Identification and Characterization of the CYP52 Family of Candida tropicalis ATCC 20336, Important for the Conversion of Fatty Acids and Alkanes to α,ω-Dicarboxylic Acids</i> , 69 APPL. ENVIRON. MICROBIOL. 10, 5983-5991 (2003).	Craft 2003	N/A
1022	Catalogue entry for NCYC 825 <i>Yarrowia lipolytica</i> (available at https://www.ncyc.co.uk/catalogue/yarrowia-lipolytica-825).	NCYC 825	N/A
1023	Gotthard Kunze et al., <i>The complete genome of Blastobotrys (Arxula) adenivorans LS3 - a yeast of biotechnological interest</i> , 7 BIOTECHNOLOGY FOR BIOFUELS 66, 1-15 (2014).	Kunze 2014	N/A

No.	Description	Referred To Herein As	Corresponding Hall-Ellis Exhibit No.
1024	W. J. Middelhoven et al., <i>Trichosporon adeninovorans</i> sp. nov., a yeast species utilizing adenine, xanthine, uric acid, putrescine and primary n-alkylamines as the sole source of carbon, nitrogen and energy, 50 ANTONIE VAN LEEUWENHOEK, 369-378 (1984).	Middelhoven 1984	N/A
1025	Cletus P. Kurtzman & Christie J. Robnett, <i>Multigene phylogenetic analysis of the Trichomonascus, Wickerhamiella and Zygoascus yeast clades, and the proposal of Sugiyamaella</i> gen. nov. and 14 new species combinations, 7 FED’N OF EUROPEAN MICROBIOL. SOC’Y., 141-151 (2007).	Kurtzman 2007	N/A
1026	Cletus P. Kurtzman et al., <i>Advances in yeast systematics and phylogeny and their use as predictors of biotechnologically important metabolic pathways</i> , 15 FEMS YEAST RESEARCH 6, 1-17 (2015)	Kurtzman 2015	N/A
1027	G. Sybren de Hoog et al., <i>Name Changes in Medically Important Fungi and Their Implications for Clinical Practice</i> , 53 J. CLINICAL MICROBIOL. 4, 1056-1062 (2015).	de Hoog 2015	N/A
1028	Heide-Marie Daniel et al., <i>On the reclassification of species assigned to Candida and other anamorphic ascomycetous yeast genera based on phylogenetic circumscription</i> , 106 ANTONIE VAN LEEUWENHOEK, 67-84 (2014).	Daniel 2014	N/A

No.	Description	Referred To Herein As	Corresponding Hall-Ellis Exhibit No.
1029	MARC-ANDRÉ LACHANCE ET AL., <i>Chapter 90: Candida Berkhout (1923)</i> , THE YEASTS: A TAXONOMIC STUDY, Vol. 2, Elsevier (5 th Ed. 2011).	Lachance 2011	N/A
1030	MAUDY TH. SMITH ET AL., <i>Chapter 87: Blastobotrys von Klopotek (1967)</i> , THE YEASTS: A TAXONOMIC STUDY, Vol. 2, Elsevier (5 th Ed. 2011).	Smith 2011	N/A
1031	James E. Bailey, <i>Toward a Science of Metabolic Engineering</i> , SCIENCE, Vol. 252, 1668-1675 (1991).	Bailey 1991	N/A
1032	Susan M. Barns et al., <i>Evolutionary Relationships among Pathogenic Candida Species and Relatives</i> , 173 J. BACTERIOL. 7, 2250-2255 (1991).	Barns 1991	N/A
1033	Susan M. Barns et al., <i>Evolutionary Relationships among Pathogenic Candida Species and Relatives</i> , 173 J. BACTERIOL. 7, Table 3 at 2252 (1991).	Barns 1991	N/A
1034	Athanasios Beopoulos et al., <i>Control of Lipid Accumulation in the Yeast Yarrowia lipolytica</i> , 74 APPL. ENVIRON. MICROBIOL. 24, 7779-7789 (2008).	Beopoulos 2008	N/A
1035	Bernard Dujon, <i>Yeast evolutionary genomics</i> , 11 NATURE GENET., 512-524 (2010).	Dujon 2010	N/A
1036	Bernard Dujon, <i>Yeast evolutionary genomics</i> , 11 NATURE REV. GENET., Figure 1 at 513 (2010).	Dujon 2010	N/A
1037	Toni Gabaldon et al., <i>Evolutionary genomics of yeast pathogens in the Saccharomycotina</i> , 16 FEMS YEAST RESEARCH 6, 1-10 (2016).	Gabaldon 2016	N/A

No.	Description	Referred To Herein As	Corresponding Hall-Ellis Exhibit No.
1038	Pannida Khunnamwong et al., <i>Description of Diutina gen. nov., Diutina siamensis, f.a. sp. nov., and reassignment of Candida catenulata, Candida mesorugosa, Candida neorugosa, Candida pseudorugosa, Candida ranongensis, Candida rugosa and Candida scorzettiae to the genus Diutina</i> , 65 INT. J. SYST. EVOL. MICROBIOL., 4701-4709 (2015)	Khunnamwong 2015	N/A
1039	Sarah E. Kidd et al., <i>Fungal Nomenclature: Managing Change is the Name of the Game</i> , OFID, 1-15 (2023).	Kidd 2023	N/A
1040	KREGER-VAN RIJ, THE YEASTS: A TAXONOMIC STUDY, Elsevier (3 rd Rev. and Enlarged Ed. 1984).	Kreger-van Rij 1984	N/A
1041	Cletus P. Kurtzman, <i>Phylogenetic circumscription of Saccharomyces, Kluyveromyces and other members of the Saccharomycetaceae, and the proposal of the new genera Lachancea, Nakaseomyces, Naumovia, Vanderwaltozyma, and Zygorulasporea</i> , 4 FEMS YEAST RESEARCH, 233-245 (2003).	Kurtzman 2003	N/A
1042	Yoann Millerioux et al., <i>Drug-resistant cassettes for the efficient transformation of Candida guilliermondii wild-type strains</i> , 11 FEMS YEAST RESEARCH, 457-463 (2011).	Millerioux 2011	N/A
1043	Nicolas Papon et al., <i>Deus ex Candida genetics: overcoming the hurdles for the development of a molecular toolbox in the CTG clade</i> , 158 MICROBIOL., 585-600, (2012).	Papon 2012	N/A

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1044	Nicolas Papon et al., <i>Emerging and Emerged Pathogenic Candida Species: Beyond the Candida albicans Paradigm</i> , 9 PLOS. PATHOG. 9, 1-4 (2013).	Papon 2013	N/A
1045	Kalaivani Paramasivan & Sarma Mutturi, <i>Progress in terpene synthesis strategies through engineering of Saccharomyces cerevisiae</i> , 37 CRIT. REV. BIOTECHNOL. 8, 974-989 (2017).	Paramasivan 2017	N/A
1046	Sung-Oui Suh et al., <i>Phylogenetics of Saccharomycetales, the ascomycete yeasts</i> , 98 MYCOLGOIA 6, 1006-1017 (2006).	Suh 2006	N/A
1047	Masako Takashima & Takashi Sugita, <i>Taxonomy of Pathogenic Yeasts Candida, Cryptococcus, Malassezia, and Trichosporon: Current Status, Future Perspectives, and Proposal for Transfer of Six Candida Species to the Genus Nakaseomyces</i> , 63 MED. MYCOL. J., 119-132 (2022).	Takashima 2022	N/A
1048	Yuuki Yamada et al., <i>Diversity and Analysis of Bacterial Terpene Synthases</i> , 515 METH. ENZYM. 7, 123-162 (2012).	Yamada 2012	N/A
1049	Ningyan Zhang & Zhiqiang An, <i>Chapter 11: Heterologous Protein Expression in Yeasts and Filamentous Fungi</i> , MANUAL OF INDUSTRIAL MICROBIOLOGY AND BIOTECHNOLOGY, 145-156 (3 rd Ed. 2010).	Zhang 2010	N/A

No.	Description	Referred To Herein As	Corresponding Hall-Ellis Exhibit No.
1050	LinkedIn Resume of Kimberly Ann Aeling	N/A	N/A
1051	LinkedIn Resume of Pierre-Yves De Wals	N/A	N/A
1052	LinkedIn Resume of Eric Scott	N/A	N/A
1053	LinkedIn Resume of Michael Walbridge	N/A	N/A
1054	LinkedIn Resume of Thomas Beardslee	N/A	N/A
1055	Curriculum Vitae of Sylvia Hall-Ellis	N/A	N/A
1056	ROBERT F. WEAVER, <i>Chapter 2: The Molecular Nature of Genes</i> , MOLECULAR BIOLOGY (McGraw Hill 5 th Ed. 2012).	N/A	1007
1057	BRUCE ALBERTS ET AL., MOLECULAR BIOLOGY OF THE CELL, Garland Science (5 th Ed. 2008).	N/A	1008
1058	Thomas J. Maimone & Phil S. Baran, <i>Modern Synthetic Efforts Toward Biologically Active Terpenes</i> , 3 N. CHEM. BIO. 7, 396-407 (2007).	N/A	1009
1059	Sam Zwenger & Chhandak Basu, <i>Plant Terpenoids: Applications and Future Potentials</i> , 3 BIOTECHNOL. MOL. BIOL. REV. 1, 1-7 (2008).	N/A	1010
1060	John Blazeck & Hal S. Alper, <i>Promoter engineering: Recent Advances In Controlling Transcription At The Most Fundamental Level</i> , 8 BIOTECHNOL. J., 46-58 (2013).	N/A	1011

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1061	Vikki M. Weake & Jerry L. Workman, <i>Inducible Gene Expression: Diverse Regulatory Mechanisms</i> , 11 NATURE REV. GENET., 426-437 (2010).	N/A	1012
1062	Katrin Weinhandl et al., <i>Carbon Source Dependent Promoters in Yeasts</i> , 13 MICROBIAL CELL FACTORIES 5, 1-17 (2014).	N/A	1013
1063	Tamotsu Kanai et al., <i>An n-Alkane-Responsive Promoter Element Found in the Gene Encoding the Peroxisomal Protein of Candida tropicalis Does Not Contain a C₆ Zinc Cluster DNA-Binding Motif</i> , 182 J. BACTERIOL. 9, 2492-2497 (2000).	N/A	1014
1064	Tamotsu Kanai et al., <i>An n-Alkane-Responsive Promoter Element Found in the Gene Encoding the Peroxisomal Protein of Candida tropicalis Does Not Contain a C₆ Zinc Cluster DNA-Binding Motif</i> , 182 J. BACTERIOL. 9, Figure 3 at 2496 (2000).	N/A	1015
1065	Yutaka Masuda et al., <i>Cloning and Characterization of the POX2 Gene in Candida maltosa</i> , 167 GENE 1-2, 157-161 (1995).	N/A	1016
1066	Yutaka Masuda et al., <i>Cloning and Characterization of the POX2 Gene in Candida maltosa</i> , 167 GENE 1-2, Figure 2 at 159 (1995).	N/A	1017

No.	Description	Referred To Herein As	Corresponding Hall-Ellis Exhibit No.
1067	Michael E. Lee et al., <i>A Highly Characterized Yeast Toolkit for Modular, Multipart Assembly</i> , 4 ACS. SYNTH. BIOL. 9, 975-986 (2015).	N/A	1018
1068	J.P. van der Walt & J.A. von Arx, <i>The Yeast Genus Yarrowia gen. nov.</i> , 46 ANTONIE VAN LEEUWENHOEK, 517-521 (1980).	N/A	1019
1069	J.P. van der Walt et al., <i>Arxula gen. nov. (Candidaceae), a new anamorphic, arthroconidial yeast genus</i> , 57 ANTONIE VAN LEEUWENHOEK, 59-61 (1990).	N/A	1020
1070	David L. Craft et al., <i>Identification and Characterization of the CYP52 Family of Candida tropicalis ATCC 20336, Important for the Conversion of Fatty Acids and Alkanes to α,ω-Dicarboxylic Acids</i> , 69 APPL. ENVIRON. MICROBIOL. 10, 5983-5991 (2003).	N/A	1021
1071	Catalogue entry for NCYC 825 <i>Yarrowia lipolytica</i> (available at https://www.ncyc.co.uk/catalogue/yarrowia-lipolytica-825).	N/A	1022
1072	Gotthard Kunze et al., <i>The complete genome of Blastobotrys (Arxula) adenivorans LS3 - a yeast of biotechnological interest</i> , 7 BIOTECHNOLOGY FOR BIOFUELS 66, 1-15 (2014).	N/A	1023

No.	Description	Referred To Herein As	Corresponding Hall-Ellis Exhibit No.
1073	W. J. Middelhoven et al., <i>Trichosporon adeninovorans</i> sp. nov., a yeast species utilizing adenine, xanthine, uric acid, putrescine and primary n-alkylamines as the sole source of carbon, nitrogen and energy, 50 ANTONIE VAN LEEUWENHOEK, 369-378 (1984).	N/A	1024
1074	Kurtzman, Cletus P., and Christie J. Robnett. "Multigene Phylogenetic Analysis of the <i>Trichomonascus</i> , <i>Wickerhamiella</i> and <i>Zygoascus</i> Yeast Clades, and the Proposal of <i>Sugiyamaella</i> gen. nov. and 14 New Species Combinations." <i>FEMS Yeast Research</i> , vol. 7, issue 1 (January 2007): 141-151.	N/A	1025
1075	James A. Sloots et al., <i>Glucose-responsive and oleic acid-responsive elements in the gene encoding the peroxisomal trifunctional enzyme of Candida tropicalis</i> , 105 GENE 1, 129-134 (1991).	Sloots 1991	N/A
1076	James A. Sloots et al., <i>Glucose-responsive and oleic acid-responsive elements in the gene encoding the peroxisomal trifunctional enzyme of Candida tropicalis</i> , 105 GENE 1, 130, Figure 1 (1991).	Sloots Fig. 1	N/A

I, Hal Alper, Ph.D., declare as follows:

I. INTRODUCTION

1. I am the Kenneth A. Kobe Distinguished Professor in the McKetta Department of Chemical Engineering and Cockrell School of Engineering at The University of Texas at Austin. I have over 20 years of experience researching and publishing on the topics of metabolic engineering, genetic engineering, synthetic biology, and engineering biology to produce organic molecules of interest such as biofuels, commodity and specialty chemicals, and protein pharmaceuticals. A significant focus of this work and my background and training has been in the area of engineering fungal systems. Moreover, a research focus during this time has included the development of fungal inducible promoters as well as the bioproduction of terpenes such as carotenoid products. To date, I have published over 170 scientific publications across this range of topics. My complete curriculum vitae is found at Annex A.

2. I understand that Patent Owner Radici Chimica S.p.A. (“Radici”) is the assignee of U.S. Patent No. 11,781,148 (the “’148 Patent”). On behalf of Petitioners LCY Biotechnology Holding, Inc. (“LCY”), I have been asked to provide expert opinions on this matter that I understand relates to whether the claims of the ’148 Patent lack enablement and/or are obvious in view of prior-art references.

3. I am over the age of 18 and otherwise competent to make this declaration. I am being compensated at my customary hourly rate of \$950.00, as well as being reimbursed for reasonable business expenses. My compensation is not contingent upon the outcome of these proceedings or the opinions I reach.

4. I understand that this declaration is being submitted in support of LCY's petitions for Post-Grant Review ("PGR") of the '148 Patent. Even though there are multiple grounds supporting the PGR, I am providing a single declaration in support of all grounds.

5. In this declaration, I provide my opinions regarding the challenged '148 Patent and claims, the prior art, the level of skill of a person having ordinary skill in the art ("POSITA"), and how the POSITA would understand the prior art. The bases and reasons for my opinions, including the references I rely upon for the opinions in this declaration, are stated herein.

6. I understand that the PGR petition will have a set of exhibit numbers. Thus, I will refer to the references by name. A chart summarizing the exhibit numbers and the short name I will use to refer to each reference is included at the end of this declaration.

7. In undertaking my analyses, I have considered the challenged '148 Patent, file history, works cited on the face page of the '148 Patent, and the materials

cited herein and/or Appendix A. I have also relied upon my education, training, and experience in the subject matter.

8. As set forth in greater detail in this declaration, a POSITA would have regarded the challenged claims of the '148 Patent as being obvious in view of the prior art and lacking enablement for at least the following reasons:

a. The challenged claims are obvious under 35 U.S.C. § 103. Specifically, a POSITA would be motivated to combine prior art references Bailey and Zhang. Bailey in view of Zhang teaches every element of the challenged claims and would yield at least one embodiment of the claimed invention with a reasonable expectation of success.

b. The challenged claims lack enablement under 35 U.S.C. § 112(a). Specifically, a POSITA would need to engage in undue experimentation to make and use the full scope of the challenged claims because the breadth of the claims encompass tens of millions of permutations of distinct, genetically modified yeasts that produce terpenes, the manipulation of complex biosynthesis pathways is inherently unpredictable art, the specification discloses only a limited number of working examples and fails to articulate how additional embodiments could be reliably generated, especially in light of the well-documented (through literature and inventor declaration) unpredictable nature of working with the disclosed species.

II. EDUCATIONAL BACKGROUND, PROFESSIONAL EXPERIENCE, AND OTHER QUALIFICATIONS

9. My research currently focuses on cellular and metabolic engineering. To this end, I serve as the Principal Investigator Laboratory for Cellular and Metabolic Engineering at The University of Texas at Austin. My lab aims to rewire cellular metabolism to produce next-generation biochemicals, polymer precursors, fuels, and pharmaceuticals. In doing so, we are addressing global challenges including sustainability, human health, circular bio-economies, and clean energy/energy security. We rewire cells by uniquely merging the areas of synthetic biology, directed evolution, and pathway engineering. To complement these efforts, we have developed many synthetic genetic toolkits, high-throughput screening techniques, evolution schemes, and culturing platforms. Our mission is to enable advanced biomanufacturing using rewired host organisms. A significant focus of our work takes place in fungal host organisms including yeasts that are and/or have been classified as *Candida*. Through our routine work, we have utilized and engineered several species (and even many of the specific strain numbers including, but not limited to ATCC MYA-2613, ATCC 20460, ATCC 20362, and ATCC 20336) listed in the '148 Patent specifications. Efforts also include the engineering of cells for the production of terpenes (such as carotenoid products) as well as the use of inducible promoters including those that are alkane and fatty-acid inducible.

10. I received my Ph.D. in 2006 from the Department of Chemical Engineering at the Massachusetts Institute of Technology. After receiving my Ph.D., I completed a two-year post-doctoral research appointment at the Whitehead Institute for Biomedical Research studying fungal genetics and also completed a one-year (overlapping) post-doctoral fellowship at Shire Human Genetic Therapies with a focus on mammalian cell genetics and cell line development.

11. I started as a faculty member at The University of Texas at Austin in 2008. My current position has the title of Kenneth A. Kobe Professorship in Chemical Engineering at The University of Texas at Austin in the McKetta Department of Chemical Engineering in the Cockrell School of Engineering. I also hold Graduate Studies Committee appointments in the Department of Molecular Biosciences and Department of Microbiology.

12. For many years, I have taught university courses that relate to topics of biochemistry, chemical engineering, and microbiology. In these courses, I cover a range of topics including biochemistry, microbial physiology, protein synthesis, protein structure, enzyme mechanisms, biodegradation, reaction kinetics, engineering biology, biochemical engineering, cellular engineering, and metabolic engineering. I regularly teach methods of engineering biology to produce organic molecules.

13. I supervise graduate, post-doctoral, undergraduate, and visiting scholar researchers in my laboratory. I have mentored graduate students in dissertation research involving a variety of topics, including synthetic biology, directed evolution and high-throughput technologies, platform strain engineering, and unique biotic-abiotic interfaces. During my career to date, I have served as PhD advisor for 41 past or current PhD students and post-doctoral research advisor for 7 past or current post-docs, and undergraduate research advisor for 110 past or current undergraduate students.

14. I have been recognized for my work through various awards, including the Andreas Acrivos Award for Professional Progress in Chemical Engineering (AIChE), the Society for Industrial Microbiology and Biotechnology Young Investigator Award, the Jay Bailey Young Investigator Award in Metabolic Engineering, the Camille Dreyfus Teacher-Scholar Award, the Biotechnology and Bioengineering Daniel I.C. Wang Award, Regents' Outstanding Teaching Award (selected by the UT System), the Office of Naval Research Young Investigator Award, the DuPont Young Professor Award, the Invited Participant, National Academies Keck Futures Initiative Conference, Synthetic Biology: Building on Nature's Inspiration, the Protégé, The 6th Annual Conference of The Academy of Medicine, Engineering & Science of Texas-attendance based on nomination by National Academy Member, the Texas Exes Teaching Award, the Camille and

Henry Dreyfus New Faculty Award, and the Merck Student/Young Investigator Poster Award for Best Poster (Metabolic Engineering Conference).

15. I have been elected as a fellow of the National Academy of Inventors, the American Association for the Advancement of Science, and The American Institute for Medical and Biological Engineering and The International Academy of Medical and Biological Engineers. These distinctions are recognitions granted to only a select few within the scientific community.

16. I have authored and co-authored more than 170 peer-reviewed research publications in journals including, but not limited to *Nature*, *Synthetic Systems and Biotechnology*, *ACS Synthetic Biology*, *Metabolic Engineering*, *Environmental Technology & Innovation*, *Current Opinion in Biotechnology*, *Materials Today Bio*, *Lab on a Chip*, *Biotechnology Advances*, *Advanced Functional Materials*, *Applied Microbiology and Biotechnology*, *Biotechnology and Bioengineering*, *Journal of Environmental Management*, *Frontiers in Microbiology*, *Nature Chemical Biology*, *Critical Reviews in Biotechnology*, *Life*, *Nature Communications*, *PNAS*, *Bioactive Materials*, *Microbial Cell Factories*, *Biotechnology Notes*, *Journal of Industrial Microbiology and Biotechnology*, *FEMS Yeast Research*, *Bioresource Technology*, *Fungal Genetics and Biology*, *Genome Announcements*, *BMC Systems Biology*, *Nucleic Acids Research*, *Nature Reviews Microbiology*, *Biotechnology and Bioengineering*, *Biofuels*, *Nature Biotechnology*, and *Chemical Engineering Science*.

Collectively, these papers have garnered over 17,000 citations. Throughout my career, I have (and in many cases continue to) serve various roles on scientific journals from Editorial Board Member to Associate Editor roles. These include prestigious journals in the field such as *Biotechnology and Bioengineering*, *Biotechnology Advances*, *Metabolic Engineering*, *Discover Chemical Engineering*, *Applied Microbiology and Biotechnology*, *Microbial Cell Factories*, *FEMS Yeast Research*, and *Synthetic and Systems Biotechnology*, among others. As such, I am intimately familiar with the rigor of the scientific peer review process.

17. I have been listed as a patent inventor on over 15 patent applications. From these, I am listed as an inventor on 9 issued US patents.

18. Since 2018, I have served as an expert where I provided expert advice regarding strain and genetic engineering (mostly in fungi) with a focus on bioproduction, genetic constructs, and rewiring metabolic flux. A list of matters upon which I have served as an expert is attached hereto as Annex B and incorporated herein.

III. APPLICABLE LEGAL STANDARDS

19. I am not an attorney, and therefore my understanding of patent law and the legal standards set forth in this report is based on explanations provided to me by counsel.

20. I understand that a patent is eligible for *post-grant* review if it contains or contained at any time a claim that has an effective filing date on or after March 16, 2013. I understand that the effective filing date is either the actual filing date of the patent or the filing date of the earlier application for which the patent is entitled to a right of priority.

21. I understand that the validity of a patent claim must be analyzed as of the “priority date” of that claim. I understand that for a patent claim to be entitled to a given priority date, the patent specification as filed on that date must adequately describe the claimed subject matter, such that the POSITA would understand that the inventor was in possession of the claimed invention as of the priority date.

22. I understand that the Patent Office may cancel issued claims of the ’148 Patent if the Petitioner demonstrates that the claims fail to satisfy the requirements of patentability that the claims must be nonobvious and that the specification must provide adequate written description and enablement support for the full scope of the invention.

23. I understand that the claims should be understood according to their ordinary and customary meaning as understood by a person of ordinary skill in the art (“POSITA”) at the time of the invention in light of the language of the claims, the specification, and the prosecution history of record. I have been provided a copy of the prosecution history of record as Exhibit 1002.

a. Obviousness

24. I understand that a patent claim is “obvious,” and thus invalid, if the differences between the subject matter sought to be patented and the prior art are such that the subject matter as a whole would have been obvious at the time the invention was made to the hypothetical POSITA in the art to which said subject matter pertains. I understand that factual determinations relevant to the obviousness inquiry include (1) the scope and content of the prior art, (2) the differences between the claimed invention and the prior art, (3) the level of ordinary skill in the art, and (4) secondary indicia of obviousness.

25. I understand that a claim is obvious if all of the claimed elements were known in the prior art and one skilled in the art would have combined the elements as claimed with a reasonable expectation of success. I understand that obviousness may also be shown by demonstrating that it would have been obvious to modify what is taught in a single piece of prior art to create the patented invention or by combining teachings of two or more references. I understand that in order for a claimed invention to be considered obvious, there must be some rational underpinning for the proposed combination.

26. I have been advised that courts recognize that a motivation to combine may be found explicitly or implicitly in the interrelated teachings of multiple patents

and/or references and the background knowledge, creativity, and common sense of the person of ordinary skill.

27. I have also been informed that, for a claim to be obvious, it is required both that (1) a skilled artisan would have been motivated to combine the teachings of the prior art references, and (2) that the skilled artisan would have had a reasonable expectation of success in doing so. I understand that a reasonable expectation of success does not, however, require absolute certainty of success.

28. I understand that a patentee may cite certain secondary considerations of nonobviousness, such as unexpected properties, failure of others, and copying, to support the nonobviousness of a challenged patent claim. I further understand that secondary considerations of nonobviousness are only relevant if there is a nexus between the merits of the claimed invention and the evidence of such secondary considerations.

29. I further understand that, in order to show nexus, a patentee must show that the evidence of secondary considerations results both from what is claimed and what is novel in the claim. I also understand that, to support nonobviousness, any secondary considerations must be reasonably commensurate with the scope of the claim that is challenged as obvious. I am not aware of any secondary considerations of nonobviousness that would support the nonobviousness of the challenged claims.

However, if Radici contends that the challenged claims are supported by one or more secondary considerations of nonobviousness, I reserve the right to respond.

b. Enablement

30. I understand that a patent claim lacks “enablement,” and thus is invalid, if the specification does not describe how to make and how to use the claimed invention. The invention that a POSITA must be enabled to make and use is that defined by the claim(s) of the particular application or patent.

31. I understand that a claim is unpatentable for lack of enablement if the specification does not contain a sufficiently full, clear, concise, and exact description of the manner and process of making and using the full scope of the claimed invention to enable a POSITA to make and use the invention without undue experimentation at the critical date. However, experimentation to make and use the invention is allowable if it is not undue.

32. I understand that in deciding whether a person having skill in the art would have to engage in undue experimentation to make and use the invention, several factors may be considered: (a) the breadth of the claims, (b) the nature of the invention, (c) the state of the prior art, (d) the level of one of ordinary skill, (e) the level of predictability in the art, (f) the amount of direction provided by the inventor, (g) the existence of working examples, and (h) the quantity of experimentation needed to make and use the invention based on the content of the disclosure.

33. I understand that no one factor is alone dispositive. Rather, whether the degree of experimentation required is undue may involve weighing these factors in the context of the claimed invention and the state of the art at the time of the original application, to determine whether a person skilled in the art would need to engage in undue experimentation to make and use the full scope of the claimed invention.

34. I understand that if a patent claims a group or “genus” of subject matter (such as a group of enzymes), a sufficient written description requires the disclosure of either a representative number of species falling within the scope of the claimed genus, or structural features that the members of the genus have in common, so that a POSITA can “visualize or recognize” the members of the genus.

IV. THE '148 Patent

35. I understand that the '148 Patent claims priority to PCT Application No. PCT/US2018/041579, filed on July 11, 2018, which claims priority to U.S. Provisional Application No. 62/532,297, filed on July 13, 2017.

36. The '148 Patent was filed as U.S. Pat. App. No. 16/630,567 (The '567 application) on January 13, 2020. The '567 application was subsequently published as US20210079408 on March 18, 2021. The '567 application issued as U.S. Patent No. 11,781,148 (the '148 Patent) on October 10, 2023.

37. The claims of the '148 Patent are generally directed to engineered cells and microorganisms capable of producing terpenes. The claims require that these

genetically modified yeasts are selected from the group consisting of *Candida* spp and *Blastobotrys* spp. The claims generally require that these genetically modified yeasts include a heterologous nucleic acid that encodes for a terpene biosynthesis polypeptide. The claims generally require that the heterologous nucleic acid is a fatty acid inducible promoter for producing terpenes.

38. For example, Claim 1 of the '148 Patent recites:

1. A genetically modified microorganism, comprising:

one or more heterologous nucleic acids encoding one or more terpene biosynthesis polypeptides, wherein expression of at least one of the heterologous nucleic acids is regulated by a nucleic acid that provides for fatty acid or alkane induction of expression of the terpene biosynthesis polypeptide:

wherein the one or more heterologous nucleic acids that encode one or more terpene biosynthesis polypeptides is selected from the group consisting of terpene synthase, phytoene synthase, geranylgeranyl diphosphate synthase, phytoene desaturase, lycopene cyclase, bifunctional lycopene cyclase/phytoene synthase, β -carotene ketolase, β -carotene hydroxylase, astaxanthin synthase, zeaxanthin glucosyltransferase and valencene synthase,

wherein the nucleic acid that provides for said fatty acid induction of expression of the terpene biosynthesis polypeptide is a promoter,

wherein the genetically modified microorganism is a yeast selected from the group consisting of *Candida* ssp [sic] and *Blastobotrys* ssp., [sic] and

wherein said genetically modified microorganism provides for terpene biosynthesis.

V. THE FILE HISTORY OF THE '148 Patent

39. I reviewed certain parts of the file history of the '148 Patent, which are cited below. For purposes of this declaration, I summarize those parts of the file history that I reviewed with the input of counsel who explained the relevant patent law. Independent patent Claim 1 was prosecuted as application Claim 163. Prosecution of the application included multiple Patent Office actions rejecting this claim and its dependent claims and rejecting the applicant's arguments attempting to obtain allowance. Eventually, after claim amendments and arguments, patentee obtained allowance.

40. On February 18, 2022, the Examiner rejected claims 163-165 under 35 U.S.C. § 103 as being unpatentable over Sharpe *et al.*, WO 2008/073367, in view of Juretzek *et al.*, *Biotechnol. Bioprocess. Eng.*, 2000. Ex. 1002, '148 PH at 2686-88. In response, Applicants argued that the cited references failed to teach or suggest "a

promoter being responsive to fatty acid or alkane induction” in the claimed organisms, that such promoter activity was the “unexpected and surprising result” of the claimed invention, and that the alleged teaching of Juretzek were insufficient to show the claimed promoter activity as the exemplary gene, beta-galactosidase, is easily activated and “a starting point” – thus not comparable to the “much more complex[] genes” claimed. *Id.* at 2905-13.

41. On July 29, 2022, the Examiner issued a Final Office Action maintaining the rejection of claims 163-165 of the application as being unpatentable over Sharpe in view of Juretzek for reasons highly similar to those provided in the Non-final Office Action and further providing counterpoints to each of the Applicant’s arguments including that the “Applicants never disclose what said results might be and why they would be unexpected” and that the limitations relied upon by the Applicant’s arguments were not present in the pending claims. *Id.* at 2922-28.

42. In response to this rejection, Applicants requested continued examination of the claims, amended Claim 163, and presented a new Claim 184 pursuant to 37 C.F.R. § 1.114. *Id.* at 2955-68. In addition, on October 20, 2022 Applicants submitted a supplemental declaration under 37 C.F.R. §1.132 of inventor Thomas Beardslee, in which Dr. Beardslee asserted that the claimed invention was distinct from the cited references because “extensive trial and error and a deep

understanding of the processes and the parameters to be checked and selected” would be needed to convert the teachings of the cited references into the claimed features. *Id.* at 2944. Moreover, Dr. Beardslee asserted that the claimed invention is to “a specific genetically engineered microorganism, while in the prior arts cited are either generic oleaginous yeasts structurally different or promoters suitable for regulating the overexpression of beta-galactosidase in the yeast *Yarrowia lipolytica*.” *Id.* at 2941-46.

43. On December 2, 2022, the Examiner entered the request for continued examination and issued a non-final rejection of claims 163-165 and 184 . *Id.* at 2971-82. The claims were rejected, among other grounds, under 35 U.S.C. § 103 as being obvious over Sharpe in view of Juretzek. *Id.* at 2972-76. Specifically, the Examiner was unpersuaded by the October 20, 2022, declaration, noting that:

Here this is not found convincing because this begs the question, given the breadth and scope of the current claims Applicant’s seemingly are arguing their own claims are not enabled? They state the cited prior art are generic to *Yarrowia* but they themselves have demonstrated specific genetically modified microorganisms capable of expressing a terpene enzyme under the control of a promoter included by fatty acids or alkanes only in *Candida*.

Id. at 2977.

44. In response to the Examiner’s rejection, on March 22, 2023, Applicants filed a response to the December 2, 2022, Office Action cancelling Claim 164 and

amending Claims 163, 165, and 184. *Id.* at 3021-29. Specifically, the Applicants amended independent Claim 163 to narrow the genetically modified microorganisms to “a yeast selected from the group consisting of *Candida* ssp. and *Blastobotrys* ssp.” *Id.* at 3022. Notably, the Applicants expressly argue that “the Examiner based the rejection on the expression of foreign proteins in the yeast *Yarrowia lipolytica* . . . however, the presently claimed invention is directed to gene expression on yeast selected from the group consisting of *Candida* ssp and *Blastobotrys* ssp.” *Id.* at 3025. Additionally, Applicants submitted a supplemental declaration of Dr. Beardslee on March 22, 2023, wherein Dr. Beardslee asserted that the amended claims obviated the § 103 rejection because the amended Claim was “now directed to the mandatory production of the specific terpene target product” in the selected yeast genera (which allegedly differed from the cited references which teach embodiments in *Yarrowia lipolytica*). *Id.* at 3032 ¶ 9. In addition, Dr. Beardslee introduced new examples (which were asserted to have been available at the time of filing but not included in the initial specification): (1) *Candida* strain sVZ287 and (2) a *Blastobotrys* strain sAA9936, both of which were asserted to generate lycopene when grown on oleic acid. *Id.* at 3033. While the latter was described with some detail in the declaration, the former strain was provided without any justification or description.

45. The Examiner allowed the claims and on October 10, 2023, the '148 Patent issued. *Id.* at 3047. In withdrawing the rejections, the Examiner noted that

“[t]he rejection of Sharpe . . . in view of Juretzek . . . is withdrawn in view of the amendments to the claims to limit the host cell to *Candida* ssp. and *Blastobotrys* ssp.”
Id. at 3045.

VI. OVERVIEW OF THE PRIOR ART

A. Bailey

46. Bailey claims priority to two United States provisional applications, U.S. Application 60/848,062 filed September 28, 2006 and U.S. Application 60/902,145 filed February 16, 2007.

47. PCT/US2007/021092 was filed September 28, 2007 and published as WO 2008/042338 on April 10, 2008.

48. Bailey introduces the significance of the described technology by explaining that “[c]arotenoids are organic pigments . . . used industrially as ingredients for food and feed stocks. . . [f]or example, astaxanthin is widely used in salmon aquaculture to provide the orange coloration characteristic of their wild counterparts.” Ex. 1003, Bailey at p. 2 [0003]. Bailey further elaborates that carotenoids have numerous known health benefits (e.g., vitamin A precursors, antioxidant properties). Ex. 1003, Bailey at p. 2 [0003]. Bailey explains that carotenoids are “hydrocarbon compounds...derived from the five-carbon compound IPP, including triterpenes (C30 diapocarotenoids) and tetraterpenes (C40 carotenoids) as well as their oxygenated derivatives and other compounds that are,

for example, C35, C50, C60, C70, C80 in length or other lengths. Ex. 1003, Bailey at p. 15 [0052].

49. However, Bailey explains that prior to its 2006/2007 patent disclosures “the biological systems that produce carotenoids are industrially intractable and/or produce the compounds at such low levels that commercial scale isolation is not practicable” and emphasizes “a need for improved biological systems that produce carotenoids.” Ex. 1003, Bailey at p. 2 [0004].

50. In describing a solution to these historical challenges, Bailey discloses “improved systems for the biological production of carotenoids and/or retinolic compounds.” Ex. 1003, Bailey at p. 2 [0005]. In one aspect, the improved biological systems of Bailey take advantage of the common knowledge that carotenoids and/or retinolic compounds are lipid (i.e., fat) soluble compounds stored within lipid bodies of the yeast cells that produce the compounds. Ex. 1003, Bailey at p. 3 [0008]. To do so, Bailey developed yeasts having large lipid bodies that serve as carotenoid storage containers. Specifically, the yeasts of Bailey are oleaginous, which is “the ability of an organism to accumulate lipid to at least about 20% of its dry cell weight.” Ex. 1003, Bailey at p. 17 [0053].

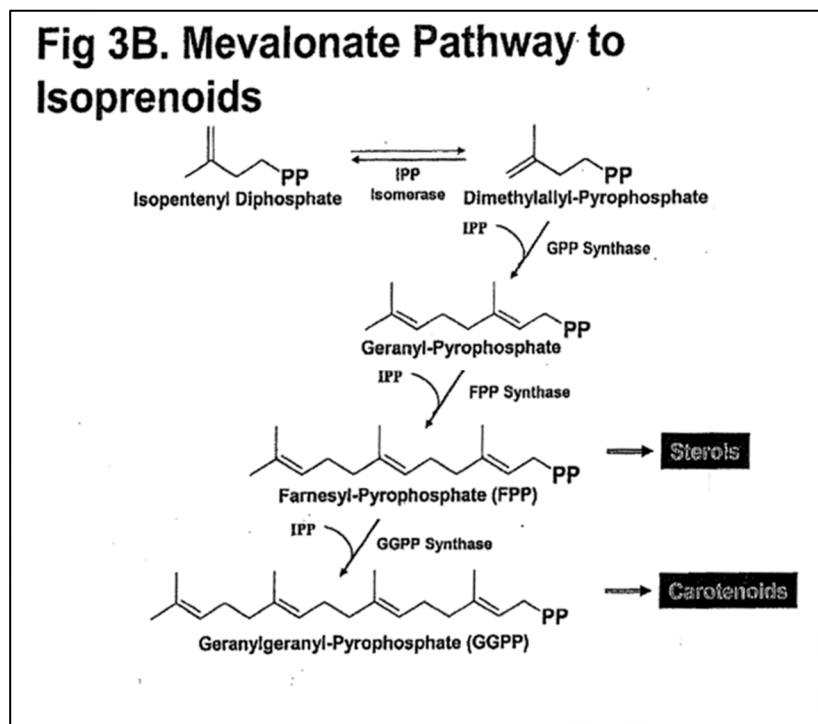
51. The yeast strains developed by Bailey are genetically modified organisms or “recombinant fung[i] . . . and produces at least one carotenoid and/or retinolic compound, . . . wherein the recombinant fungus is a member of a genus

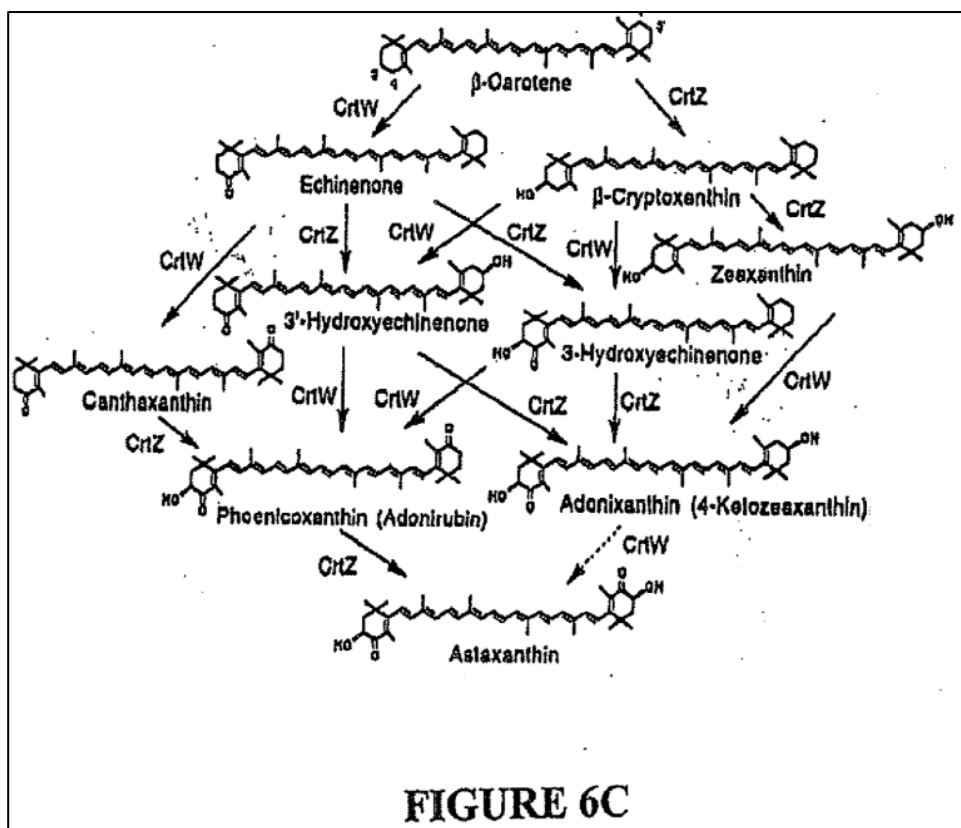
selected from the group consisting of: *Aspergillus*, *Blakeslea*, *Botrytis*, *Candida*, . . . *Trichoderma*, *Trichosporon* . . . wherein the recombinant fungus comprises at least one modification as compared with a parental fungus, the at least one modification being selected from the group consisting of . . . carotenogenic modifications . . . and wherein the at least one modification . . . confers to the recombinant fungus the ability to produce the at least one carotenoid . . .” Ex. 1003, Bailey at p. 5 [0013].

52. By way of example, Bailey describes multiple species examples of recombinant fungi, such as those from the genus *Candida*. For example, Bailey discloses “a genetically modified microorganism,” with respect to 1[preamble] and further recites “...the recombinant fungus is a member of a genus selected from...*Candida*,” and that “the recombinant fungus...is of a species selected from the group consisting of... *Candida pulcherrima*, *Candida revkaufi*, *Candida tropicalis*, *Candida utilis*” Ex. 1003, Bailey at p. 6 [0014]. Specific details on the constructions and utility of these genetically modified strains are described by Bailey in Examples 1-8, 10, 12, 16. Ex. 1003, Bailey at p. 77 [00273].

53. Some of the genetically modified yeasts of Bailey take advantage of well-established biosynthetic pathways, including the mevalonate pathway involved in the biosynthesis of isoprenoids shown in FIG. 3B and the biosynthesis of astaxanthin from β -carotene as shown in FIG. 6C. For example, Bailey provides large tables (e.g., Tables 14, 15, 17a, 17b, 18, 19, 20, 21, 23, 24) of enzymes used in

their genetically modified yeasts for producing carotenoids and/or retinolic compounds.





54. Related to the pathways shown in FIGS. 3B and 6C, Bailey specifically describes “genetically modified *Y. lipolytica* strain...” having “increased activity or expression of GPP synthase, increased activity or expression of FPP synthase, ...increased activity or expression of GGPP synthase, decreased activity or expression of GGPP synthase.” Bailey at p. 10 [0023]. Further, Bailey describes genetically modified yeasts “that have been engineered to express at least one carotenoid hydroxylase (e.g., CrtZ) polypeptide...to produce zeaxanthin, lutein, β-cryptoxanthin, and/or astaxanthin.” Bailey at p. 60 [00195]. While describing other modified yeasts, Bailey also describes strains “that have been engineered to express at least one carotenoid ketolase (e.g., CrtO/CrtW) polypeptide in combination with

at least one carotenoid hydroxylase (e.g., CrtZ) polypeptide.” Ex. 1003, Bailey at p. 10 [00196].

55. Besides having large carotenoid storage containers, the genetically modified yeasts of Bailey “produce carotenoid(s) and/or retinolic compound(s) at a level at least about 1%, and in some embodiments at least about 3-20%, of their dry cell weight,” which is higher than unmodified yeasts. Ex. 1003, Bailey at p. 29 [0082]. Thus, the yeast of Bailey provides for a practicable and scalable biological source for carotenoids and/or retinolic compounds.

56. To enhance carotenoid production capabilities, Bailey genetically modifies the yeasts by introducing heterologous nucleic acids that encode for a number of carotenoid biosynthesis polypeptides. Ex. 1003, Bailey at p. 48 [0147]. These carotenoid biosynthesis polypeptides are responsible for producing carotenoids. For example, by introducing these heterologous nucleic acids, Bailey is “increasing production of a naturally-produced carotenoid, for example by increasing the level and/or activity of one or more proteins involved in the synthesis of that carotenoid and/or by decreasing the level or activity of one or more proteins involved in a competing biosynthetic pathway.” Ex. 1003, Bailey at p. 47 [0146].

57. The genetically modified yeasts of Bailey also incorporate fatty acid inducible promoters to express the heterologous genes related to carotenoid production. In fact, Bailey discloses a list of “promoters and/or regulatory sequences

that may be employed in expression of polypeptides” “for the biological production of carotenoids and/or retinolic compounds” which includes the acyl-CoA oxidase 4 (POX4) and hydratase-dehydrogenase epimerase (HDE) promoters, but does not expressly disclose that the POX4 or HDE promoters are fatty-acid and/or alkane inducible. Ex. 1003, Bailey at p. 36 [00108] and p. 2 [0005].

58. Bailey recites enhanced carotenoid and/or retinolic compound production in a number of common yeast genus including *Aspergillus*, *Blakes/ea*, *Botrytis*, *Candida*, *Trichoderma*, and *Trichosporon*, while providing working examples in *Yarrowia lipolytica*. Ex. 1003, Bailey at p. 5 [0013] and p. 92 [00344]. It is worthwhile to note that despite contemplating several across several broad genus as above, Bailey teaches preferences of using only select strains within the genus (see Claim 3 of Bailey as an example) and ultimately shows working examples in *Y. lipolytica*.

B. Zhang

59. Zhang claims priority to United States provisional application No. 60/401,212 filed August 5, 2002.

60. U.S. Patent Application No. 10/629,266 was filed July 29, 2003, and published as U.S. Patent Publication No. 2004/0265980 on December 30, 2004.

61. The technology developed by Zhang is directed to the “use of the POX4 gene promoter as well as other promoters from other alkane or fatty acid inducible

genes, for the expression of heterologous genes in yeast cells.” Ex. 1004, Zhang at p. 15 [0013]. To accomplish this, Zhang genetically modified yeast cells to include fatty acid inducible promoters that express heterologous genes related to chemical compound production. Ex. 1004, Zhang at p. 15 [0014]. For example, as shown on p. 16 [0019], Zhang discloses the use of POX4, a fatty acid or alkane inducible gene promoter, to express genes in yeast strains across various genera such as “*Yarrowia*, *Candida*, *Bebaromyces*, *Saccharomyces*, *Schizosaccharomyces*, or *Pichia*,” for producing chemical compounds. *Id.* It is worthwhile to note that despite contemplating a broad genus such as *Candida*, Zhang teaches using only select strains within the general genus of *Candida* (Ex. 1004, Zhang at p. 30 at Claim 14) and ultimately teaches a specific strain containing a β -oxidation modification (Ex. 1004, Zhang at p. 30 at Claim 16), likely owing to its known breadth as a genus. Beyond POX4, Zhang demonstrated through MS/MS experimentation “other *Candida* genes which have inducible promoters which may be used in the methods of the present invention.” Ex. 1004, Zhang at p. 23 [0078]. Included in this list were additional promoters (disclosed in Ex. 1004, Zhang at p. 23 (Table 11)) such as those regulating the hydratase-dehydrogenase epimerase protein as evinced by Spot Numbers MSN 1092 and MSN 1093. The ’148 Patent identifies the hydratase-dehydrogenase epimerase protein as HDE. Ex. 1001 ’148 Patent at p. 70 (20:2-3), (referencing the “promoter region of a gene encoding *Candida* hydratase-

dehydrogenase-epimerase (HDE).”). This was well-established in the field and would have been known by a POSITA. *See* Ex. 1075, Sloots 1991 in its entirety.

62. Besides the various yeast genera disclosed above, Zhang focuses on yeast strains from the genus *Candida*, including “*C. tropicalis*, *C. maltosa*, *C. apicola*, *C. paratropicalis*, *C. albicans*, *C. cloacae*, *C. guilliermondii*, *C. intermedia*, *C. lipolytica*, *C. parapsilosis*, or *C. zeylenoides*.” Ex. 1004, Zhang at p. 3 [0019]. Zhang even provides working examples (see *id.*, Zhang at p. 21 at examples 4, 7, 8 and at p.22 at Table 9) for a POX4-initiated enzyme production in H5343, a strain of *C. tropicalis*.

63. Zhang provides descriptions and examples of how to prepare genetically modified yeast strains to include exogenous genes having fatty acid or alkane inducible gene promoters. Ex. 1004, Zhang at p. 16 [0022]. For example, Zhang discloses steps of isolating a fatty acid or alkane inducible gene promoter from one yeast, linking the promoter to an open reading frame of a target gene involved in a target chemical compound production to create a fusion gene, inserting the fusion gene into an expression vector, transforming a different yeast host cell with the expression vector, and then culturing the transformed yeast host cell in media to produce the target chemical compound. Ex. 1004, Zhang at p. 16 [0022].

64. By way of example, Zhang explicitly describes creating a POX4 promoter-gene fusion for various gene types, which were then all successfully

integrated individually into *Candida tropicalis*. Zhang at Examples 1-6. Zhang then successfully demonstrated that these genetically modified strains each had a significant chemical compound production rate over the wildtype yeast strains. Ex. 1004, Zhang at p. 22 [0075]-[0076].

65. Additionally, Zhang provides many examples of both saturated and unsaturated fatty acids that can be used to induce their promoters. For example, on p. 19 [0053], Zhang describes “[e]xamples of saturated fatty acids which may be applied to yeast cells incorporating the aforementioned fusion constructs according to the present invention include caproic, enanthic, caprylic, pelargonic, capric, undecylic, lauric, myristic, pentadecanoic, palmitic, margaric, stearic, arachidic, behenic acids and combinations thereof. Examples of unsaturated fatty acids which may be applied to genetically modified yeast cells include palmitoleic, oleic, erucic, linoleic, linolenic acids and combinations thereof.” Similarly, Zhang discloses a number of alkanes that can be used to induce their promoters. Specifically, Zhang describes “alkanes and fractions of alkanes may be applied which include chain links from C12 to C24 in any combination.” Ex. 1004, Zhang at p. 19 [0053].

VII. THE QUALIFICATIONS AND BACKGROUND OF THE PERSON OF ORDINARY SKILL IN THE ART

66. I understand that in patent law, a POSITA is a hypothetical person to whom the challenged patent is directed. I have been informed that the factors for determining the level of ordinary skill in the art may include the educational level of

the inventors of the challenged patent; the type of problems encountered in the art; the prior-art solutions to those problems; the rapidity with which innovations are made; the sophistication of the technology; and the educational level of active workers in the field. I further understand that the challenged patent's purpose may be a relevant consideration. I have considered these factors in my analysis.

67. The '148 Patent is directed to biological methods for producing terpenes and engineered cells, and microorganisms capable of such production. Ex. 1001, '148 Patent at p. 61 (1). The inventors of the '148 Patent include Kimberly Ann Aeling, Ph.D; Pierre-Yves De Wals; Eric Scott, Ph.D.; Michael Walbridge, and Thomas Beardslee, Ph.D. *Id.* Kimberly Ann Aeling obtained her Ph.D. in Biological Sciences in 2007 from UC Irvine. Ex. 1050. Pierre-Yves De Wals obtained a Master of Science in 2007 from Université de Montréal. Ex. 1051. Eric Scott obtained a Ph.D. in bioinformatics in 2015 from UC San Diego. Ex. 1052. Michael Walbridge obtained a Masters in microbiology in 1997 from The University of Texas. Ex. 1053. Thomas Beardslee obtained a Ph.D. in biochemistry in 2000 from the University of Nebraska-Lincoln. Ex. 1054.

68. The type of problems encountered in the art include the unpredictable nature of complex biosynthesis pathway manipulations; fungal genetics and *Candida* phylogeny; heterologous promoter architecture and function, the organization of organisms and gene sequencing; and uncertainty in being able to

produce something new in a fungal host organism. The prior art solutions to those problems include the demonstration of promoter elements; the demonstration and establishment of terpene pathways; the genetic modification of yeasts; and genetic construct design, as further discussed herein.

69. Regarding the “rapidity with which innovations are made,” around the 2017 timeframe, this was an era of rapid growth with lots of research activity and startups focused on small molecule based biological production. A full expansion of the field was underway with complex examples buoyed by the benefits of gene synthesis and sequencing. Regarding the “sophistication of the technology,” this area is quite sophisticated and often required a customized solution for each strain engineering application. Fully realized strain engineering that solved industrially important problems at the time still required an extensive number of years of PhD-level research to achieve results. The technology involves a nuanced combination of science and art to identify new solutions.

70. Regarding the “educational level of active workers in the field,” active workers would typically have a Ph.D. or a Masters of Science with multiple years of experience. Workers with a Bachelor’s of Science degree would likely be heavily mentored or would be taking on the role of a technician—as opposed to working individually on strain design and discovery.

71. Thus, it is in my opinion that a POSITA would have knowledge and experience regarding protein biochemistry, genetic modification of yeasts, understanding of general genetics and phylogeny, designing synthetic biology constructs, producing small molecules with yeasts, isolating these small molecules, enzyme activity and expression, and characterization of nucleic acid sequences. The POSITA would be able to apply these concepts to genetic modification of yeasts to produce small molecules. The POSITA would therefore have an advanced degree (e.g., Ph.D. or Masters) in a relevant discipline (e.g., chemical, biological, or microbiological field) and several years of advanced academic (e.g., post-doctoral or higher) and/or professional experience in a relevant discipline, or the equivalent in years of experience.

72. Under this definition, I am qualified to offer opinions regarding the claims of the '148 Patent. I have over 20 years of experience research and publishing on genetic modification of yeasts for producing small molecules, including extensive analysis and working knowledge of nucleic acids and fatty acid inducible promoters to use as tools within these yeasts. I am familiar with the tools involved with genetically modifying yeasts for these purposes for an array of yeast strains.

VIII. CLAIM CONSTRUCTION

73. I have been advised that in this *Post-Grant* Review, the claim terms generally should be given their ordinary and customary meaning as a POSITA would

have understood them at the time of the invention and in light of the patent and its prosecution history. Furthermore, I have been instructed that a claim's plain language can help guide the interpretation of the claim terms in question, and the manner in which a term is used in a claim may be probative of its meaning.

74. I also understand that because the meaning of a claim term as understood by a POSITA is often not immediately apparent, and because patentees frequently use terms idiosyncratically, the court looks to public sources that show what a person of skill in the art would have understood disputed claim language to mean. I understand those sources include the "intrinsic evidence", *i.e.*, the patent itself, including the claims, the specification and, if in evidence, the prosecution history (or file history).

75. I understand that in a *Post-Grant* Review proceeding, only claim terms that are actually in dispute need to be construed. Thus, terms that are not in dispute, or those for which alternative constructions would not render a different finding, do not need to be construed.

76. I have been informed that if the specification reveals a special definition given to a claim term by the patentee that differs from the meaning it would otherwise possess, the inventor's lexicography governs. Given that the claim terms at issue in the challenged claims are defined by the '148 Patent specification itself, it is my opinion that no further claim construction is required.

IX. OPINIONS AS TO INVALIDITY OF THE '148 Patent

A. Claims 1-3 are unpatentable under post-AIA 35 U.S.C. §103 as obvious over Bailey and Zhang

1. Claim 1

a. 1[preamble]: “A genetically modified microorganism, comprising:”

77. The '148 Patent describes a “genetic modification” and “genetically modified” microorganism as:

a “genetically modified” or “genetically engineered” cell, organism or microorganism is one in which the genetic make-up of the cell, organism or microorganism has been modified. Genetic modification encompasses a variety of alterations and can be accomplished in numerous ways. A genetic modification includes, but is not limited to, any of the following alterations: modification of the expression of an endogenous gene (e.g., the amount, pattern, timing and/or regulation (e.g., inducibility) of expression of a gene), disruption or deletion of an endogenous gene, increasing the copy number of an endogenous gene, mutation of an endogenous gene (including the regulatory components, exons, introns and/or peptide- or protein-encoding portions of a gene), and introduction of heterologous nucleic acid in to a cell or cells.

Ex. 1001, '148 Patent at p. 96 (71:62-72:9).

As a POSITA, I believe this is to be an accurate and unambiguous definition of “genetic modification” as well as “genetically modified microorganism” and reflects the understanding that the genetic framework of the microorganism (e.g., the

deoxyribonucleic acid (DNA)) has been altered. Because of this, it is my understanding that claim interpretation is not necessary for this term.

78. The preamble is disclosed in Bailey. Bailey discloses “recombinant fung[i] . . . and produces at least one carotenoid and/or retinolic compound, . . . wherein the recombinant fungus is a member of a genus selected from the group consisting of: *Aspergillus*, *Blakeslea*, *Botrytis*, *Candida*, . . . *Trichoderma*, *Trichosporon* . . . wherein the recombinant fungus comprises at least one modification as compared with a parental fungus, the at least one modification being selected from the group consisting of . . . carotenogenic modifications . . . and wherein the at least one modification . . . confers to the recombinant fungus the ability to produce the at least one carotenoid . . .” Ex. 1003, Bailey at p. 13 [0013].

79. As discussed in ¶¶ 51 and 78, a POSITA would understand that a “recombinant fungus” is a fungal organism which has had its DNA / genetic make-up altered in some fashion (such as to incorporate DNA from another source) and fits within the definition of a “genetic modification.” Moreover, a POSITA would have understood that “at least one modification as compared with a parental fungus” as described in Bailey defines the scope of the “recombinant fungus” and unequivocally matches the definition of a “genetic modification” as described in the preamble of the ’148 Patent. Ex. 1001, ’148 Patent at p. 290 at Claim 1 (preamble).

80. As discussed in paragraph [0013] of Bailey, the fungal genera described as being suitable organisms to generate recombinant fungi, including *Candida* and *Trichosporon*, are microorganisms. Ex. 1003, Bailey at p. 5 [0013].

81. Accordingly, in teaching recombinant fungi Bailey discloses “a genetically modified microorganism.”

b. 1[a]: “one or more heterologous nucleic acids encoding one or more terpene biosynthesis polypeptides,”

82. The ’148 Patent defines a “heterologous nucleic acid” as being a nucleic acid that is “non-native” to a given cell and more specifically states:

The term “heterologous,” “exogenous” or “foreign” as used herein with respect to a composition or quality (e.g., a gene, nucleic acid, peptide, polypeptide, cellular activity, genetic composition, gene expression, and the like) refers to the composition or quality not being a physically existing part or attribute of a reference cell, organism or microorganism. For example, a heterologous, exogenous or foreign nucleic acid can be any nucleic acid that is introduced into a cell or microorganism as part of a genetic modification of the cell or microorganism. A heterologous, exogenous or foreign composition (such as, for example, a nucleic acid) includes compositions that may be identical to an endogenous composition (e.g., a nucleic acid gene sequence that is introduced into a cell or microorganism to increase the copy number and/or alter the positioning or expression of the same nucleic acid sequence existing therein) or may be different from an endogenous composition.

Ex. 1001, '148 Patent at p. 96 (72:60-73:10).

The '148 Patent further reiterates this definition along with synonyms when it states:

The terms “heterologous polynucleotide,” “heterologous nucleic acid,” “exogenous polynucleotide,” “exogenous nucleic acid,” “foreign polynucleotide” and “foreign nucleic acid,” used interchangeably herein, refer to a polynucleotide as it relates to a particular reference cell or organism (e.g., a host cell or organism) and is one that is not present in the genetic composition of the reference cell or organism. A heterologous polynucleotide includes a polynucleotide that may be identical in nucleotide sequence to an endogenous polynucleotide present in a cell, but if introduced into the cell would alter the genetic composition of the cell by, for example, increasing the copy number of the polynucleotide in the cell, altering the position(s) of the polynucleotide in the cell genome, altering the expression of the polynucleotide in the cell, and the like. Thus, such a heterologous nucleic acid thereby genetically modifies the cell into which it is introduced.

Ex. 1001, '148 Patent at p. 130 (140:49-65).

83. A POSITA would understand that nucleic acids are the building blocks of deoxyribonucleic acid (“DNA”) and can include both coding (*i.e.*, genes) and non-coding (*e.g.*, regulatory) sequences. *See* Ex. 1007, Weaver 2012 at p. 5 (Chapter 2). The '148 Patent further clarifies that a heterologous nucleic acid is broadly defined

as a nucleic acid sequence that deviates from the native DNA sequence and can include a: (a) mutated endogenous promoter, (b) modification of the endogenous promoter (*e.g.*, by directed mutagenesis), and (c) replacement of a promoter with a promoter from another genome or another section of the same genome. Ex. 1001, '148 Patent at p. 69-70 (18:66-19:15). Through this definition, the heterologous DNA piece need not be completely foreign in nature to this organism, just placed into the genome in a manner such that its introduction would alter the genetic composition of the cell compared to the reference cell. Furthermore, a POSITA would understand that the '148 Patent uses the term “endogenous” to mean “originating from an organism”, in a manner that matches its plain and ordinary meaning in the common lexicon. A POSITA would understand that a “heterologous nucleic acid” of the '148 Patent can encompass a chemical, physiological, or genetic modification of a native DNA sequence that results in a change to the DNA sequence such that it differs from the native DNA sequence. *See* Ex. 1001, '148 Patent at p. 70 (19:8-10) (stating “[a] modification may be performed by directed mutagenesis, and the mutated promoter may be referred to as ‘heterologous’ because it is no longer native”). A POSITA would also understand that a “heterologous nucleic acid” of the '148 Patent can encompass an endogenous DNA sequence used in a non-native fashion such as to genetically modify the cell. *See* Ex. 1001, '148 Patent at p. 130 (140:55-59) (stating “A heterologous polynucleotide includes a polynucleotide that

may be identical in nucleotide sequence to an endogenous polynucleotide present in a cell, but if introduced into the cell would alter the genetic composition of the cell”).

84. The '148 Patent explains that “[a] cell synthesizes native proteins, including enzymes, in vivo based on the sequence of deoxyribonucleic acid (DNA) encoding the protein.” Ex. 1001, '148 Patent at p. 62 (3:21-24). A POSITA would understand “encoding” to mean that the DNA sequence codes for the RNA responsible for synthesis of a polypeptide (e.g., a protein). This principle is well understood as the “Central Dogma of Biology.” *E.g.*, Ex. 1008, Alberts 2008 at p. 78.

85. The '148 Patent further defines “terpene biosynthesis polypeptide” as “any polypeptide capable of producing a terpene molecule or a terpene precursor.” Ex. 1001, '148 Patent at p. 69 (17:31-32).

86. The '148 Patent explains that terpenes are compounds generally made from isoprene units (C_5H_8). Ex. 1001, '148 Patent at p. 89 (58:31-32). Further, the '148 Patent describes how “units of isoprene...can be joined together in a variety of different combinations to generate thousands of terpene compounds.” Ex. 1001, '148 Patent at p. 89 (58:31-35). A POSITA would understand that “terpene” is an over-arching term for a large class of natural products that are generally categorized based on their number of chemical building blocks, or isoprene units. Because of the broad definition of terpenes, encompassing all molecules having the

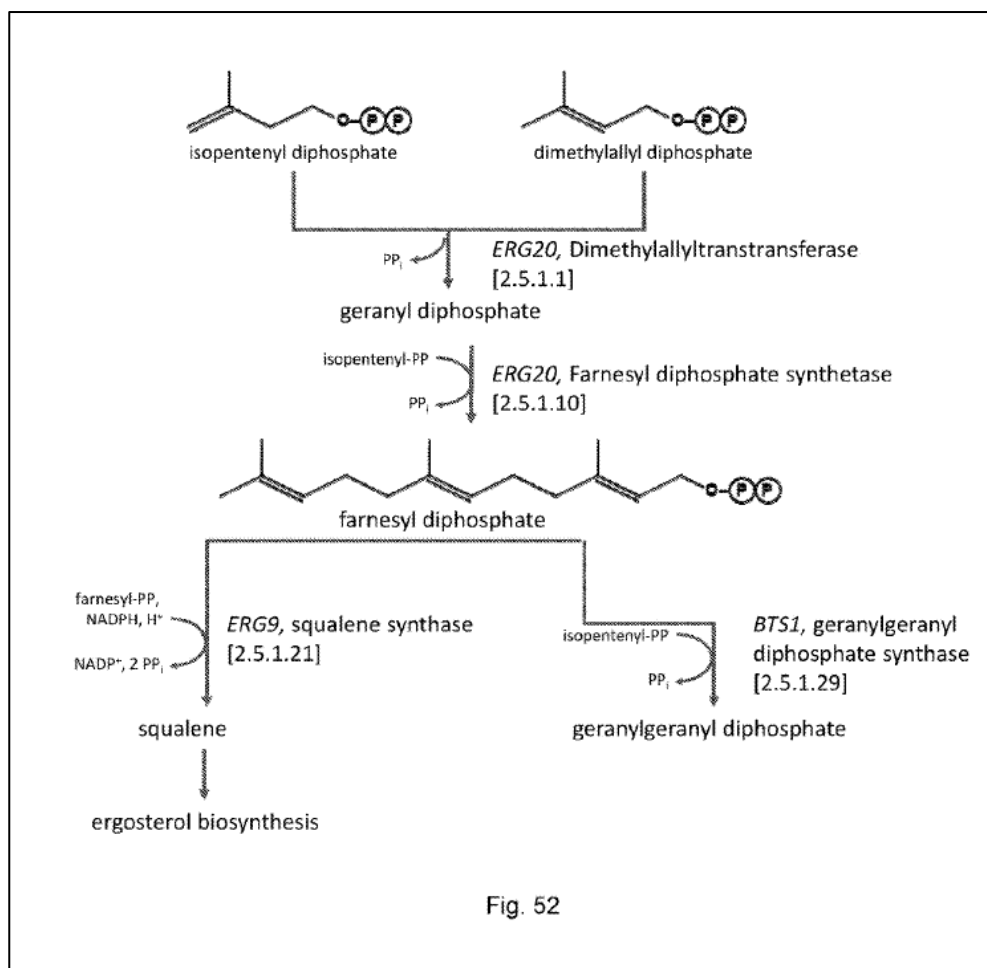
isoprene unit building block structure, there is no finite number of possible molecules, but a POSITA would understand the term “terpene” to encompass an extraordinarily broad class of distinct, natural product molecules. The extreme breadth and diversity of this molecule is evident in the scientific literature and a POSITA would recognize that even counting the number of possible species is challenging in such a broad class with estimates ranging at no fewer than 25,000 distinct terpenes (*e.g.*, Ex. 1010, Zwenger 2008 at p. 1 (col. 2, lines 5-9)) to as many as 55,000 distinct terpenes (*e.g.*, Ex. 1009, Maimone 2007 at p. 1 (Abstract)). The '148 Patent further explains that “[t]erpenes may include carotenoids” and that “[o]ver 700 carotenoids are known, which include, for example, carotenes (*e.g.*, γ -carotene, β -carotene).” Ex. 1001, '148 Patent at p. 69 (17:4-9).

87. Because a carotenoid is a type of terpene and because a terpene biosynthesis polypeptide is a polypeptide “capable of producing a terpene molecule or a terpene precursor”, a POSITA would understand that a carotenoid biosynthesis polypeptide is a “terpene biosynthesis polypeptide.” Ex. 1001, '148 Patent at p. 69 (17:31-32).

88. Moreover, the '148 Patent describes that a terpene biosynthesis polypeptide may include: “a terpene synthase, a phytoene synthase, a geranylgeranyl diphosphate synthase, a phytoene desaturase, a lycopene cyclase, a bifunctional lycopene cyclase/phytoene synthase, a β -carotene ketolase, a β -carotene

hydroxylase, a astaxanthin synthase, a zeaxanthin glucosyltransferase, and a valencene synthase.” Ex. 1001, ’148 Patent at p. 69 (17:32-40). Each of these are proteins (*i.e.*, polypeptides) that function as enzymes within the carotenoid biosynthesis pathway. As a result, these would be recognized by a POSITA as carotenoid biosynthesis polypeptides that can be broader defined as a terpene biosynthesis polypeptide.

89. As shown below, Figure 52 of the ’148 Patent illustrates an example pathway by which isoprene-based precursor molecules (*e.g.*, isopentenyl diphosphate) may undergo a number of enzymatic transformations across multiple steps and involving multiple enzymes (*e.g.*, geranylgeranyl diphosphate synthase) to generate any number of downstream products in the terpene family.



Ex. 1001, '148 Patent at p. 55 (Figure 52).

90. Figure 53 of the '148 Patent (shown below) illustrates an alternative example pathway by which isoprene-based precursor molecules (*e.g.*, farnesyl pyrophosphate) may undergo a number of enzymatic transformations across multiple steps and involving multiple enzymes (*e.g.*, lycopene cyclase) to generate any number of downstream carotenoid products in the terpene family (*e.g.*, β -carotene, a carotenoid molecule). Specifically, Figure 53 illustrates the well-known pathway by which: (1) farnesyl pyrophosphate is converted into geranylgeranyl pyrophosphate via the enzymatic activity of geranylgeranyl diphosphate synthase

(*e.g.*, CrtE); (2) geranylgeranyl pyrophosphate is converted into phytoene via the enzymatic activity of phytoene synthase (*e.g.*, CrtB); (3) phytoene may be converted into lycopene via the enzymatic activity of phytoene desaturase (*e.g.*, CrtI); (4) lycopene may be converted into β -carotene via the enzymatic activity of lycopene cyclase (*e.g.*, CrtY); (5) β -carotene may be converted into canthaxanthin via the enzymatic activity of beta-carotene mono-ketolase (*e.g.*, CrtW); and (6) canthaxanthin may be converted into astaxanthin via the enzymatic activity of a carotene hydroxylase gene (*e.g.*, CrtZ). Ex. 1001, '148 Patent at p. 56 (Figure 53).

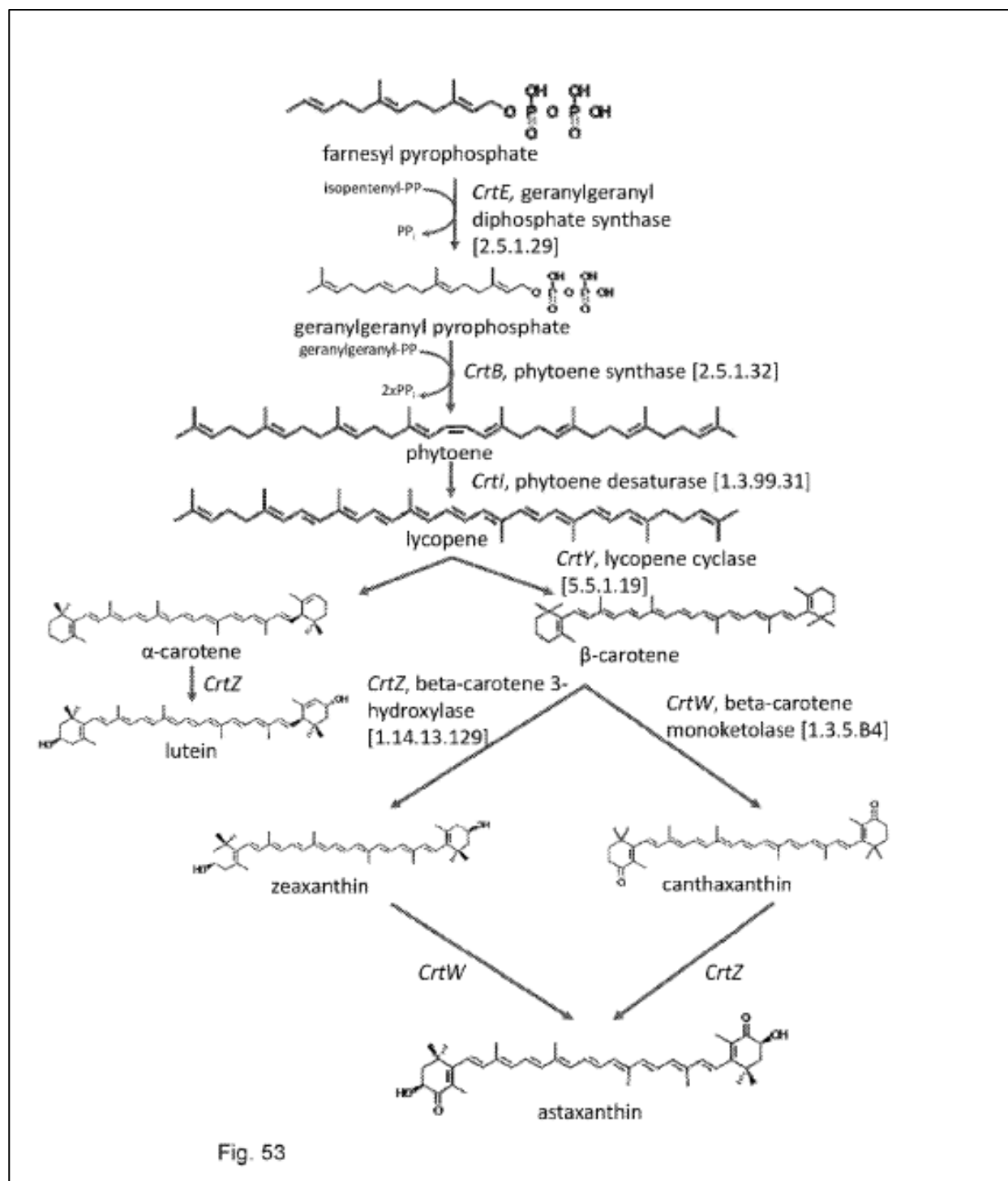


Fig. 53

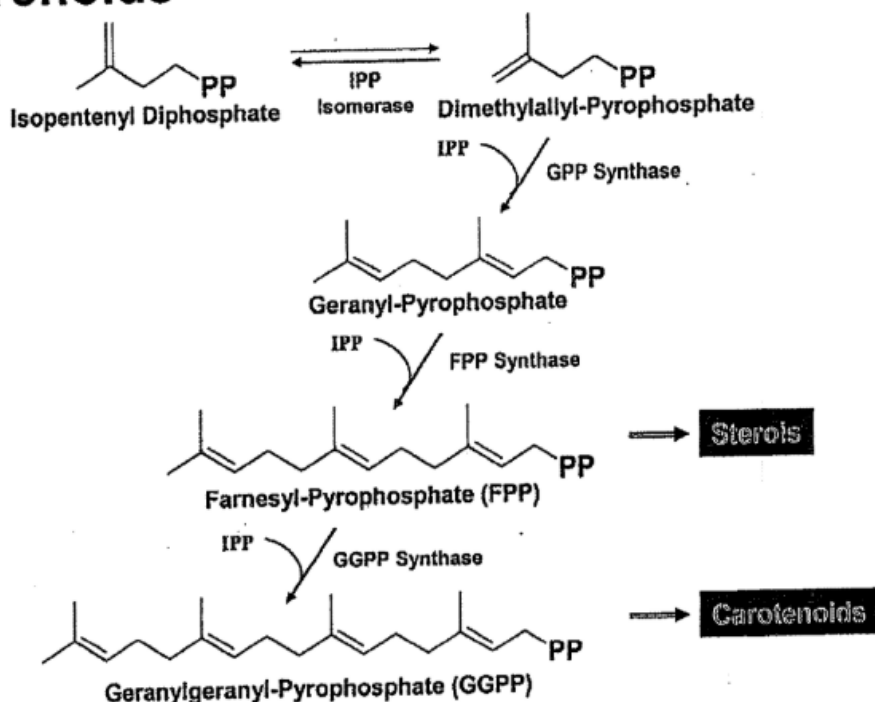
Ex. 1001, '148 Patent at p. 56 (Figure 53).

91. When giving examples of terpene biosynthesis polypeptides, the '148 Patent specifically recites "β-carotene ketolase" and "β-carotene hydroxylase." Ex.

1001, '148 Patent at p. 69 (17:30-37). Since β -carotene is an example of a carotenoid (i.e., a terpene), as a POSITA, I understand that “ β -carotene ketolase” and “ β -carotene hydroxylase” are both “terpene biosynthesis polypeptides.”

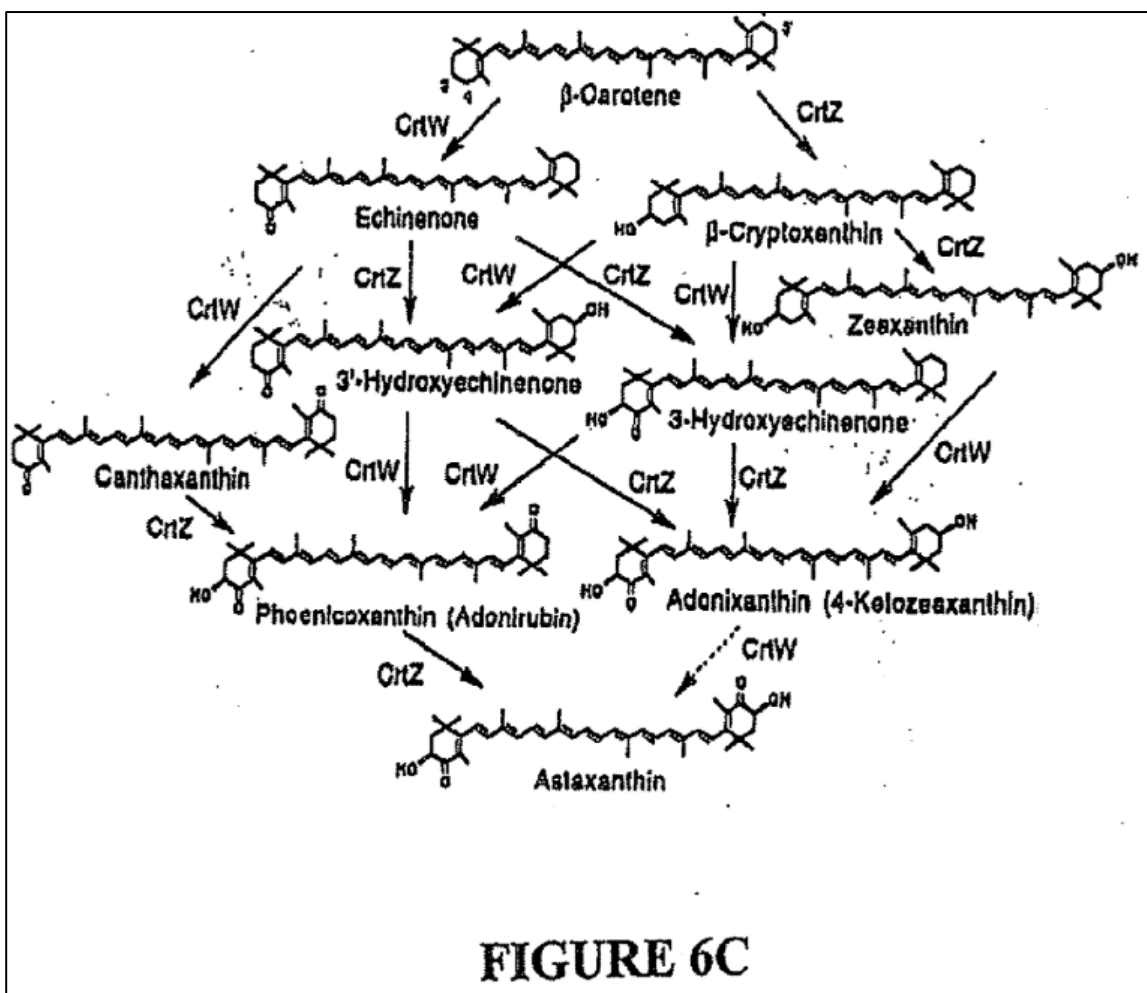
92. As discussed above, the terpene biosynthesis pathways illustrated in Figures 52 and 53 of the '148 Patent were well-known in the art by the priority date of the '148 Patent. For example, Bailey disclosed a similar illustration to that found in Figure 52 of the '148 Patent in its Figure 3B. Specifically, Figure 3B of Bailey illustrates the same known biosynthetic conversion pathways as Figure 52 of the '148 Patent, with isoprene-based precursor molecules (*e.g.*, isopentenyl diphosphate) undergoing a number of enzymatic transformations across multiple steps and involving multiple enzymes (*e.g.*, GPP synthase) to generate any number of downstream products in the terpene family (*e.g.*, carotenoids). Ex. 1003, Bailey at p. 432 (Figure 3B).

Fig 3B. Mevalonate Pathway to Isoprenoids



Ex. 1003, Bailey at p. 432 (Figure 3B).

93. Likewise, Figure 6C of Bailey (shown below) illustrates the β -carotene to astaxanthin pathway shown in Figure 53 of the '148 Patent with more realistic details highlighting the promiscuity of many of the enzymes in this pathway. A POSITA would understand that enzymes within the terpene biosynthesis pathways are not exclusive to a single isoprene-based molecule and can instead opportunistically convert various isoprene-based molecules resulting in a number of different transformation products.



Ex. 1003, Bailey at p. 437 (Figure 6C).

94. For example, the simplified Figure 53 from the '148 Patent implies that expressing only CrtZ in the presence of β -carotene yields zeaxanthin, that expressing only CrtW in the presence of β -carotene yields canthaxanthin, and that astaxanthin can be produced by simply expressing both β -carotene hydroxylase (CrtZ) and β -carotene ketolase (CrtW) in the presence of β -carotene. In reality, the illustration of the β -carotene to astaxanthin pathway in Figure 6C of Bailey shows how two terpene biosynthesis enzymes, CrtW and CrtZ, catalyze multiple and overlapping substrates

along the pathway, each with different effect. This means that temporal changes in the relative expression of CrtW and CrtZ, for example, may favor an alternative pathway and/or the accumulation of subproducts such as phoenicoxanthin instead.

95. Moreover, Bailey discloses numerous tables containing a multitude of examples of specific terpene biosynthesis polypeptides. Each table provides dozens of examples of each type of terpene biosynthesis polypeptide-spanning multiple organisms of origin and including accession information by which a POSITA could locate the specific sequence in the publicly available literature. Specifically, Bailey discloses: phytoene synthase (Ex. 1003, Bailey at p. 168 (Table 15) and p. 191 (Table 18)) (*e.g.*, CrtB), phytoene desaturase (*Id.* at p. 176 (Table 17a), p. 186 (Table 17b), and p. 191 (Table 18)) (*e.g.*, CrtI), lycopene cyclase (*Id.* at Tables p. 176 (Table 17a), p. 191 (Table 18), and p. 205 Table 23)) (*e.g.*, CrtY), β -carotene ketolase (*Id.* at p. 176 (Table 17a) and p. 198 (Table 19)) (*e.g.*, CrtW), β -carotene hydroxylase (*Id.* at p. 198 (Table 19) and p. 201 (Table 20)) (*e.g.*, CrtZ), astaxanthin synthase (*Id.* at p. 203 (Table 21)) (*e.g.*, CrtS), geranylgeranyl pyrophosphate (or diphosphate) synthase (*Id.* at p. 165 (Table 14) and p. 168 (Table 15)) (*e.g.*, CrtE), and zeaxanthin glucosyltransferase (*Id.* at p. 209 (Table 24)) (*e.g.*, CrtX).

96. In the same manner, Bailey discloses genetically modified strains engineered to alter the expression of various endogenous terpene biosynthesis polypeptides such as those that convert isopentenyl diphosphate, a fundamental

terpene building block, to carotenoids. Ex. 1003, Bailey at p. 42 [00131]. A POSITA would understand that the isopentenyl pyrophosphate referenced in Bailey is another name for isopentenyl diphosphate. For example, Bailey recites “the present invention provides a genetically modified *Y. lipolytica* strain...” having “increased activity or expression of GPP synthase, increased activity or expression of FPP synthase, ...increased activity or expression of GGPP synthase, decreased activity or expression of GGPP synthase.” Ex. 1003, Bailey at p. 9 [0023]. Thus, Bailey also discloses examples related to heterologous nucleic acids that do not rely solely on endogenous DNA sequences.

97. Accordingly, a POSITA would understand that Bailey clearly discloses “one or more heterologous nucleic acids encoding one or more terpene biosynthesis polypeptides” as claimed in 1[a] of the ’148 Patent.

c. **1[b]: “wherein expression of at least one of the heterologous nucleic acids is regulated by a nucleic acid that provides for fatty acid or alkane induction of expression of the terpene biosynthesis polypeptide:” and**

1[d]: “wherein the nucleic acid that provides for said fatty acid induction of expression of the terpene biosynthesis polypeptide is a promoter,”

98. The ’148 Patent claims a genetically modified microorganism “wherein expression of at least one of the heterologous nucleic acids is regulated by a nucleic acid” and “wherein the nucleic acid...is a promoter.” A POSITA would understand that expression of genes can be regulated by specialized non-coding DNA sequences

(i.e., “regulatory elements”) and that promoters are a specific type of transcriptional regulatory element. *E.g.*, Ex. 1008, Alberts 2008 at p. 78.

99. A POSITA would understand that promoters recruit transcription machinery and regulatory factors thereby promoting initiation of the transcription of specific portions of DNA (i.e., protein coding genes) into a messenger ribonucleic acid (“mRNA”). *E.g.*, Ex. 1008, Alberts 2008 at p. 78. The resulting mRNA may then be translated into a polypeptide (e.g., a terpene biosynthesis polypeptide). *Id.* As mentioned above, this fundamental information flow forms the basis for the “Central Dogma of Biology.”

100. Claim feature 1[b] of the ’148 Patent requires that the claimed DNA expression (i.e., the expression of at least one of the heterologous nucleic acids) is “regulated by a nucleic acid that provides for fatty acid or alkane induction.” The ’148 Patent does not expressly define this claim element, but does include descriptions of the “inducible system” at p. 70 (19:16-20:27). Further, the ’148 Patent describes these particular elements as:

Transcription regulatory elements, including promoters, for some genes are responsive to the carbon source available to the cells. For example, transcription of some genes is subject to glucose repression in which the gene may not be expressed, or is less expressed, in the presence of glucose. Thus, in contrast to unregulated constitutive promoters, transcription regulatory elements for genes such as these are repressed, derepressible and/or inducible by varying carbon sources.

Ex. 1001, ’148 Patent at p. 97 (74:45-53).

101. A POSITA would likewise understand that some promoters are “inducible” meaning that they are activated and initiate transcription of the protein coding genes into mRNA only when activated by a specific stimulus (i.e., an activator). Ex. 1008, Alberts 2008 at p. 222. Inducible promoters were well known by POSITA as of the priority date of the ’148 Patent. *E.g.*, Ex. 1012, Weake 2010; Ex. 1011, Blazek 2013, and Ex. 1013, Weinhandl 2014. In this regard, the basic mechanisms of activation and/or repression were known, a substantial body of work was reported as to cataloguing function, and these promoters were demonstrated for synthetic function when used in a heterologous fashion. Moreover, a POSITA at the priority date of the ’148 Patent would have well-understood the use of both constitutive and inducible promoters as well as the advantages of inducible promoters to specifically regulate expression, link expression to an exogenous molecule, and/or link expression to a natively produced endogenous molecule (such as lipids in oleaginous organisms) in an effort to optimize pathway performance.

102. As of the priority date of the ’148 Patent, a POSITA would also know that some inducible fungal promoters are activated by the specific activators- fatty acids and/or alkanes. *See, e.g.*, Ex. 1004, Zhang; Ex. 1014, Kanai 2000; Ex. 1016, Masuda 1995. The ’148 Patent describes that a fatty acid activator may be any number of saturated or unsaturated fatty acids including: “oleic acid, palmitoleic acid, erucic acid, linoleic acid, palmitic acid, caproic acid, undecylic acid, lauric acid,

myristic acid, pentadecanoic acid, margaric acid, stearic acid, arachidic acid, behenic acid, tridecylic acid, and linoleic acid.” Ex. 1001, ’148 Patent at p. 70 (19:24-30). The ’148 Patent describes alkane activators as being selected from hexane, heptane, nonane, decane, undecane, dodecane, tridecane, tetradecane, pentadecane, hexadecane, heptadecane, and octadecane. Ex. 1001, ’148 Patent at p. 70 (20:7-11). A POSITA would understand these recitations of fatty acid and alkane activators as being well-known in the art at the time of the ’148 Patent. For example, in 2000, Kanai understood the prevalence of inducible upstream activating sequence (“UAS”) elements responsible for the alkane induction (a mixture of decane, undecane, dodecane, tridecane), as well as, the prevalence of promoter elements responsive to n-alkane and oleic acid induction, such as POX4 and POX18 in *Candida tropicalis*. Ex. 1015, Kanai at p. 5 (Fig. 3). Even prior to this, Masuda in 1995 recognized the inducible nature of promoters such as POX2 in the presence of oleic acid based on Northern blot analysis. Ex. 1017, Masuda at p. 159 (Fig. 2). Thus, a POSITA would have easily understood the presence and prevalence of alkane and fatty acid-inducible promoters.

103. A POSITA would have clearly understood the state-of-the-art with respect to utilizing heterologous promoters to drive the expression of downstream genes. In 2017, a POSITA would have understood the prevalence of gateway cloning vectors that had fixed promoters of interest and multi-cloning sites to generically

drive expression of a coding region *E.g.*, Ex. 1018, Lee 2015. Likewise, a POSITA would have understood the introduction of an inducible promoter upstream of a coding region of interest would serve to operably link these components and conditionally drive the expression of the specific gene sequence. A POSITA would have also understood the ability to place a desired promoter upstream of a gene sequence through routine recombinant DNA technology and gene synthesis. As such, a POSITA would have expected a fatty acid / alkane promoter to perform its predictable function and inducibility irrespective of the linked, downstream coding region.

104. In addition, a POSITA would have been aware at the time of the '148 Patent that fatty acid and alkane inducible promoters had been inserted into *Candida* spp. and *Blastobotrys* spp. Specific examples of fatty acid and alkane inducible promoters were previously identified in specific/particular species such as *Candida tropicalis* (*see* Ex. 1004, Zhang and Ex. 1014, Kanai 2000) and *Candida maltosa* (*see* Ex. 1016, Masuda 1995).

105. The '148 Patent describes that the POX4 promoter is fatty acid-inducible. Ex. 1001, '148 Patent at p. 177 (234:19-20).

106. Bailey discloses a list of “promoters and/or regulatory sequences that may be employed in expression of polypeptides [] for the biological production of carotenoids and/or retinolic compounds” which includes the POX4 promoter, but

does not expressly disclose that the POX4 promoter is fatty-acid and/or alkane inducible. Ex. 1003, Bailey at p. 36 [00108] and p. 2 [0005].

107. However, in listing the POX4 promoter, Bailey expressly references the earlier published Zhang. Ex. 1003, Bailey at p. 36 [00108] (“promoter sequences from POX4 genes (see, for example, US application 2004-0265980),” where US application 2004-0265980 is Zhang). Zhang teaches that the HDE promoter is induced by fatty acids and that the POX4 promoter is induced by both fatty acids and alkanes. Ex. 1004, Zhang at p. 15 [0013], [0015], p. 16 [0022], p. 17 [0035], p. 23 (Example 10, Table 10) and p. 30 (Claims 24, 26). As recognized by the ’148 Patent and corroborated by Sloots 1991, the HDE promoter is responsible for driving the expression of the hydratase dehydrogenase epimerase gene referred to in Zhang. Ex. 1001, ’148 Patent at p. 70 (20:2-3) (discussing the “promoter region of a gene encoding Candida hydratase-dehydrogenase-epimerase (HDE).”); Ex. 1075, Sloots 1991 at p. 2. Moreover, in addition to the teaching in Zhang that the HDE promoter is induced by fatty acids, the reference of Sloots 1991 explicitly reports HDE promoter as being inducible by oleic acid and would have been known to a POSITA in the field as early as 1991. Ex. 1076, Sloots Fig. 1.

108. As laid in out in ¶¶ 25-27, I understand from legal counsel that the legal standard for a finding of obviousness requires (1) that a skilled artisan would have

been motivated to combine the teachings of the prior art references and (2) that the skilled artisan would have had a reasonable expectation of success in doing so.

109. When reading Bailey's teachings, Bailey implies—if not expressly signals—that the teachings of Zhang are necessary to Bailey. Therefore, a POSITA would have been motivated to combine the teachings of Bailey and Zhang to understand that POX4 and HDE are suitable fatty-acid promoters to induce expression by a terpene biosynthesis polypeptide (e.g., carotenoid).

110. Further a POSITA would have had a reasonable expectation of success in combining the teachings of Bailey and Zhang because both Bailey and Zhang use similar *Candida* species (as defined in the '148 Patent) in their working examples. As discussed in ¶¶ 121-123, the '148 Patent claims the invention in *Candida* species. The '148 Patent specifically states that: “[i]n some embodiments, suitable *Candida* species include, but are not limited to...*Candida lipolytica*,...*Candida tropicalis*.” Ex. 1001, '148 Patent at p. 93 (66:39-55). A POSITA would recognize that *Candida lipolytica* was reclassified as *Yarrowia lipolytica* in 1980 and is known in the art under both its current and former naming conventions. See Ex. 1019, van der Walt 1980. This is, in fact, recognized in the '148 Patent as it repeatedly references “*Yarrowia lipolytica* (formerly classified as *Candida lipolytica*).” '148 Patent at p. 93 (65:18-19); p. 93 (65:64-65); and p. 94 (68:2-3). Therefore, given the '148 Patent's definition of *Candida* species, a POSITA would understand that both *C.*

lipolytica and *C. tropicalis* are exemplary *Candida* species as claimed in the '148 Patent.

111. Paragraph [0019] of Zhang teaches that the “host cell comprising a nucleic acid molecule for a POX4 gene promoter operably linked to the open reading frame of a gene encoding a heterologous protein” can be a *Candida* species and further teaches that such *Candida* species include *C. tropicalis* and *C. lipolytica*. Further, Zhang shows examples of POX4-initiated enzyme production in various strains of *C. tropicalis*, including strain H5343. Ex. 1004, Zhang at p. 21 (Example 4), p. 22 (Example 7), p. 22 (Example 8), and p. 22 (Table 9). *C. tropicalis* strain H5343 is also identified as a beta oxidation-blocked strain of ATCC 20962. Ex. 1004, Zhang at p. 18 [0043]. This strain designation equivalency would have been known to a POSITA as it was also common in the literature including “*C. tropicalis* ATCC 20962 (initially designated strain H5343), an acyl-coenzyme A oxidase-deficient strain blocked for β -oxidation” *E.g.*, Ex. 1021, Craft 2003 at p. 2.

112. Thus, a POSITA would recognize that the *C. tropicalis* strain that the '148 Patent suggests as an exemplary embodiment of the invention is the same strain disclosed in Zhang's working examples, *C. tropicalis* strain H5343. Ex. 1001, '148 Patent at p. 93 (66:52-53). Further, a POSITA would recognize that the parent strain of *C. tropicalis* strain H5343, ATCC 20962, which as noted in ¶ 93 is

also taught by Zhang, is also used and disclosed in the '148 Patent in p. 177 (Example 2), p. 188 (Example 14), and p. 189 (Table 7).

113. Similarly, not only does Bailey use *Y. lipolytica* (also formally known as *C. lipolytica*) in its working examples of terpene production (Ex. 1003, Bailey at p. 77-105 (Examples 1-8), p 107 (Example 10), p. 111 (Example 12), and p. 123 (Example 16)), but Bailey also uses one of the specific strains that the '148 Patent lists as an example of a strain that could be used in the invention claimed in the '148 Patent. Ex. 1003, Bailey at p. 77 [00273]. Specifically, in Table 26, Bailey describes the various *Y. lipolytica* strains used in the examples including NRRL Y-1095, which a POSITA would recognize as another name for an exemplary strain of *Y. lipolytica*, ATCC-8661, which was deposited in the American Type Culture Collection (ATCC) and from there further deposited as NCYC 825 in the National Collection of Yeast Cultures by ATCC in December 1975. Ex. 1022, NCYC 825. This particular strain, as an example, has been readily available to researchers in the field dating back to at least the 1970s. *Id.* The strain has been referenced based on its ATCC number in more than 90 research publications prior to the priority date of the '148 Patent based on a Google Scholar search. Moreover, the NRRL Y-1095 name for this strain likewise appears in more than 50 research publications prior to the priority date of the '148 Patent upon a simple Google Scholar search.

114. The '148 Patent specifically lists ATCC 8661 as an exemplary embodiment of a *Y. lipolytica* strain. Ex. 1001, '148 Patent at p. 93 (66:9). As discussed in ¶¶ 110 and 123, given that the '148 Patent claims *C. lipolytica* (also known as *Y. lipolytica*) as an exemplary *Candida* species, Bailey not only discloses a genus claimed by the '148 Patent, but shows an example of a specific strain claimed by the '148 Patent.

115. Given that both Bailey and Zhang show working examples using specific strains claimed by the '148 Patent, a POSITA would have a more than reasonable expectation of success in combining the teachings of Bailey and Zhang to yield the invention claimed in the '148 Patent.

116. Because (a) Bailey teaches the use of inducible promoters (e.g., POX4 and HDE promoters) in driving the expression of heterologous nucleic acids (e.g., recombinant fungi including carotenogenic mutations) and (b) Zhang teaches that the HDE promoter is fatty acid inducible and that the POX4 promoter is both fatty-acid and alkane inducible, and (c) a POSITA would have been motivated to combine Bailey and Zhang for the reasons described above, the '148 Patent claim elements 1[b] and 1[d] are disclosed by a combination of Bailey and Zhang.

- d. **1[c]: “wherein the one or more heterologous nucleic acids that encode one or more terpene biosynthesis polypeptides is selected from the group consisting of terpene synthase, phytoene synthase, geranylgeranyl diphosphate synthase, phytoene desaturase, lycopene cyclase, bifunctional lycopene cyclase/phytoene synthase, β -carotene ketolase, β -carotene**

hydroxylase, astaxanthin synthase, zeaxanthin glucosyltransferase and valencene synthase,”

117. As discussed above in ¶¶ 82 and 85 with respect to Claim element 1[a], the '148 Patent defines “heterologous nucleic acid” as a “non-native” DNA sequence and defines “terpene biosynthesis polypeptide” as “any polypeptide capable of producing a terpene molecule or a terpene precursor.” Claim element 1[c] further requires that the specific “non-native” DNA sequence that encodes “a polypeptide capable of producing a terpene molecule or a terpene precursor” is one of following: a terpene synthase, a phytoene synthase, a geranylgeranyl diphosphate synthase, a phytoene desaturase, a lycopene cyclase, a bifunctional lycopene cyclase/phytoene synthase, a β -carotene ketolase, a β -carotene hydroxylase, a astaxanthin synthase, a zeaxanthin glucosyltransferase, and a valencene synthase.

118. As discussed above in ¶¶ 95 and 97, Bailey discloses “one or more heterologous nucleic acids encoding one or more terpene biosynthesis polypeptides” including lengthy lists of specific sequences spanning multiple organisms: phytoene synthase (Ex. 1003, Bailey at p. 191 (Table 18)), phytoene desaturase (*Id.* at p. 176 (Table 17a)), lycopene cyclase (*Id.* at p. 191 (Table 18) and p. 205 (Table 23)), β -carotene ketolase (*Id.* at p. 198 (Table 19)), β -carotene hydroxylase (*Id.* at p. 201 (Table 20)), astaxanthin synthase (*Id.* at p. 203 (Table 21)), and zeaxanthin glucosyltransferase (*Id.* at p. 209 (Table 24)). Bailey also provides working

examples of genetically modified *Y. lipolytica* strain having geranylgeranyl diphosphate synthase modifications. *Id.* at p. 10 [0023].

119. Additionally, Bailey teaches genetically modified strains “that have been engineered to express at least one carotenoid hydroxylase (e.g., CrtZ) polypeptide...to produce zeaxanthin, lutein, β -cryptoxanthin, and/or astaxanthin.” Ex. 1003, Bailey at p. 60 [00195]. Moreover, Bailey teaches genetically modified strains “that have been engineered to express at least one carotenoid ketolase (e.g., CrtO/CrtW) polypeptide in combination with at least one carotenoid hydroxylase (e.g., CrtZ) polypeptide.” *Id.* at p. 60 [00196].

120. A POSITA would understand that in disclosing genetically modified yeast strains having one or more of the specific terpene biosynthesis polypeptides recited in Claim element 1[c] of the '148 Patent and further in providing extensive lists of corresponding sequences spanning numerous organisms, Bailey teaches or suggests element 1[c]. While these genetic compositions in Bailey are demonstrated for the yeast *Y. lipolytica*, a POSITA would have had motivation to copy and some reasonable expectation of success for other yeasts such as *Blastobotrys* given that this microorganism (when newly sequenced in 2014) showed closest alignment to *Y. lipolytica*. See Ex. 1023, Kunze 2014. The concept of expressing a heterologous nucleic acid to obtain a desired enzyme function (regardless of the degree of activity)

would have been well known to a POSITA by the priority date of the '148 Patent, and found the backbone of the recombinant DNA field.

- e. **1[e]: “wherein the genetically modified microorganism is a yeast selected from the group consisting of *Candida* spp and *Blastobotrys* spp., and”**

121. The '148 Patent provides an extensive laundry list of “[y]east that . . . can be a modified organism” including the ten (10) genera cited below:

Yarrowia yeast (e.g., *Y. lipolytica* (formerly classified as *Candida lipolytica*)), *Candida* yeast (e.g., *C. revkaii*, *C. viswanathii*, *C. pulcherrima*, *C. tropicalis*, *C. utilis*), *Blastobotrys* (formerly classified as *Arxula*) (e.g., *Blastobotrys adeninivorans* (formerly classified as *Arxula adeninivorans*), *Blastobotrys mokoenaii*), *Rhodotorula* yeast (e.g., *R. glutinus*, *R. graminis*), *Rhodospiridium* yeast (e.g., *R. toruloides*), *Saccharomyces* yeast (e.g., *S. cerevisiae*, *S. bayanus*, *S. pastorianus*, *S. carlsbergensis*), *Cryptococcus* yeast, *Trichosporon* yeast (e.g., *T. pullans*, *T. cutaneum*), *Pichia* yeast (e.g., *P. pastoris*) and *Lipomyces* yeast (e.g., *L. starkeyii*, *L. lipoferus*).

Ex. 1001, '148 Patent at p. 93 (65:14-66:59).

122. Claim element 1[e] of the '148 Patent further requires the genetically modified microorganism to be a yeast selected from either of *Candida* spp and *Blastobotrys* spp. While the '148 Patent does not further define *Blastobotrys*, it does defines “*Candida* spp.” as a *Candida* species. Ex. 1001, '148 Patent at p. 93 (66:39-40). Similarly, a POSITA would understand that the term “*Blastobotrys* spp.” broadly refers to any species of the genus *Blastobotrys*. Nonetheless, by being “a

yeast” selected from *Candida* spp. and *Blastobotrys* spp., a POSITA would understand that the claim is referring to a species of yeast selected from either *Candida* spp. or *Blastobotrys* spp.

123. As discussed above in ¶¶ 51-54, Bailey discloses “a genetically modified microorganism,” with respect to 1[preamble] and further recites “...the recombinant fungus is a member of a genus selected from...*Candida*,” Ex. 1003, Bailey at pp. 5-6 [0013]. Based on this disclosure, a POSITA would understand “member” as describing a “species” of *Candida*. Bailey sets forth *Candida* species that are the exact same yeast species relied upon by the ’148 Patent. For example, in p. 6 [0013], Bailey recites “the recombinant fungus...is of a species selected from the group consisting of... *Candida pulcherrima*, *Candida revkaufi*, *Candida tropicalis*, *Candida utilis*,” which match the yeast species used in the ’148 Patent in p. 93 (66:39-55). Further as discussed in ¶ 110, the ’148 Patent defines *Candida* species as including *C. lipolytica*, (also called *Y. lipolytica*). Not only does Bailey use a *Candida* species (as defined by the ’148 Patent in its working examples of terpene production (Ex. 1003, Bailey at p. 77-105 (Examples 1-8), p. 107 (Example 10), p. 111 (Example 12), and p. 123 (Example 16)) but Bailey also uses one of the specific strains that the ’148 Patent lists as an example of a strain that could be used in the invention claimed in the ’148 Patent (Ex. 1003, Bailey at p. 77 [00273]). Therefore,

Bailey discloses a genetically modified microorganism that is a yeast selected from *Candida* spp. as defined by the '148 Patent claims and specifications.

124. Similarly, Claim 1[e] of the '148 Patent requires the “genetically modified microorganism” to be selected from the genus “*Blastobotrys* spp.” As mentioned above in ¶ 122, a POSITA would understand that the term “*Blastobotrys* spp.” broadly refers to a species of the genus *Blastobotrys*.

125. The genus *Blastobotrys* has been known by other names by the scientific community in the last decades. *Blastobotrys* was originally known under the genus name *Trichosporon* (Ex. 1024, Middelhoven 1984) and later renamed under genus name of *Arxula* (Ex. 1024, Middelhoven 1984; Ex. 1020, van der Walt 1990). It was only in 2007 that it was renamed *Blastobotrys*. Ex. 1024, Middelhoven 1984; Ex. 1025, Kurtzman 2007. A POSITA at the priority date of the '148 Patent would thus understand that any reference to *Trichosporon* or *Arxula* is an interchangeable reference to *Blastobotrys*.

126. On p. 5 [0013], Bailey discloses various *Trichosporon* yeast species, including *Trichosporon cutaneum* and *Trichosporon pullans*. A POSITA would recognize that the term “*Blastobotrys* ssp [sic]” broadly includes any species of the genus *Blastobotrys* or, by extension, *Trichosporon*. Accordingly, a POSITA would understand that the “recombinant fungus” of the genus *Trichosporon* described in Bailey is a genetically modified yeast of *Blastobotrys* spp.

127. In teaching yeast strains from *Candida* and *Trichosporon* (i.e., *Blastobotrys*), Bailey discloses “wherein the genetically modified microorganism is a yeast selected from the group consisting of *Candida* ssp and *Blastobotrys* ssp.” Therefore, Bailey discloses the same yeast genera recited in Claim 1[e].

f. 1[f]: “wherein said genetically modified microorganism provides for terpene biosynthesis.”

128. As discussed in ¶¶ 51, 78-81 above, a POSITA would understand that the “recombinant fungus” of Bailey is a “genetically modified organism” as defined by the ’148 Patent.

129. As discussed in ¶¶ 86-87, a POSITA would understand that terpenes are a broad category of compounds which includes carotenoids.

130. A POSITA would understand “provides for terpene biosynthesis” of Claim element 1[f] to mean that a terpene (e.g., carotenoid) pathway is expressed such as to allow for this molecule to be synthesized.

131. As discussed in ¶¶ 78-81 and 95-97, a POSITA would understand that in teaching a recombinant fungus configured to synthesize carotenoids, Bailey teaches the genetically modified microorganism [which] provides for terpene biosynthesis” of Claim element 1[f] of the ’148 Patent.

2. Claim 2

a. 2[a] “The microorganism of claim 1, wherein the fatty acid is a saturated fatty acid or an unsaturated fatty acid selected from one or more of oleic acid, palmitoleic acid, erucic acid,

linoleic acid, palmitic acid, caproic acid, enanthic acid, caprylic acid, pelargonic acid, capric acid, undecylic acid, lauric acid, myristic acid, pentadecanoic acid, margaric acid, stearic acid, arachidic acid, behenic acid, tridecylic acid, methyl pentadecanoate, ethyl laurate, ethyl PFAD and linolenic acid,”

132. Claim 2 depends from Claim 1, which is obvious over Bailey in view of Zhang as described above in ¶¶ 77-131. Similarly, element 2[a] is also obvious over Bailey in view of Zhang.

133. Element 2[a] includes a list of specific fatty acids used to induce activation of a fatty acid inducible promoter for expressing a terpene biosynthesis polypeptide. As described above in ¶¶ 106-107, Bailey describes POX4 as a useful promoter while citing to Zhang, while Zhang further describes POX4 as a fatty acid-induced gene promoter used to express terpene biosynthesis polypeptide. Further, in p. 19 [0053], Zhang specifically recites 18 out of the 23 fatty acids claimed in element 2[a] as examples of fatty acids that can be applied to yeast cells to induce POX4. Specifically, Zhang recites: oleic, palmitoleic, erucic, linoleic, palmitic, caproic, enanthic, caprylic, capric, undecylic, lauric, myristic, pentadecanoic, margaric, stearic, arachidic, behenic, and linolenic acids.

Examples of saturated fatty acids which may be applied to yeast cells incorporating the aforementioned fusion constructs according to the present invention include caproic, enanthic, caprylic, pelargonic, capric, undecylic, lauric, myristic, pentadecanoic, palmitic, margaric, stearic, arachidic, behenic acids and combinations thereof. Examples

*of **unsaturated fatty acids** which may be applied to genetically modified yeast cells include **palmitoleic, oleic, erucic, linoleic, linolenic acids** and combinations thereof.*

Ex. 1004, Zhang at p. 19 [0053] (*emphasis added*).

134. In addition to the recognition that Bailey in view of Zhang teaches a fatty acid-induced gene promoter used to express terpene biosynthesis polypeptide as discussed in ¶¶ 106-116, a POSITA would recognize that the majority of fatty acids claimed in feature 2[a] are taught in Zhang. Therefore, Bailey in view of Zhang teaches or suggests the features of Claim feature 2[a].

- b. 2[b] “and wherein the alkane is selected from one or more of hexane, heptane, nonane, decane, undecane, dodecane, tridecane, tetradecane, pentadecane, hexadecane, heptadecane, and octadecane.”**

135. Element 2[b] relates to element 1[b] that recites “wherein expression of at least one of the heterologous nucleic acids is regulated by a nucleic acid that provides for fatty acid or alkane induction of expression” (*emphasis added*). Given that the fatty acid and alkane elements are required only in alternative, it is my understanding that it is not necessary for a prior art reference or combination of references to meet both elements 2[a] and 2[b] to find the claim obvious.

136. Nonetheless, Bailey in view of Zhang teach or suggest 7 out of the claimed 12 alkanes as being usable for inducing gene promoters, including POX4. Specifically, in p. 19 [0053], Zhang recites that “alkanes and fractions of alkanes

may be applied which include chain links from C12 to C24 in any combination,” describing alkanes that may be applied to yeast cells to induce POX4. A POSITA would recognize that alkanes are identified by the number of their carbon chain links and that the alkanes claimed in Claim 2 of the ’148 Patent are being referenced using their International Union of Pure and Applied Chemistry (IUPAC) nomenclature. A POSITA would understand that an alkane is named by the number of carbons in its parent chain. For example, the IUPAC name for an alkane with 12 carbons is dodecane. The IUPAC names for alkanes of various chain lengths are provided in Table 1 below.

TABLE 1

IUPAC Name	Carbon Chain Length
dodecane	C12
tridecane	C13
tetradecane	C14
pentadecane	C15
hexadecane	C16
heptadecane	C17
octadecane	C18

137. A POSITA would also understand that the “chain links from C12 to C24” described in p. 19 [0053] of Zhang is a reference to the number of carbons in the parent chain of the referenced alkane. Thus, as illustrated by the above table, a POSITA would recognize that each of the alkanes claimed in Claim 2 of the ’148

Patent are identified in Zhang's description of alkane chain lengths (e.g., C12 to C24).

138. A POSITA would recognize that Zhang teaches or suggests the claimed alkanes of dodecane, tridecane, pentadecane, hexadecane, heptadecane, and octadecane. Therefore, Bailey in view of Zhang teaches or suggests the features of Claim feature 2[b].

139. Given that Bailey in view of Zhang teach or suggest each and every feature of dependent Claim 2, Claim 2 is obvious over a combination of these references.

3. Claim 3

- a. **3[a] “The microorganism of claim 1, wherein the nucleic acid promoter that provides for said fatty acid induction of expression of said terpene biosynthesis polypeptide is selected from the group consisting of hydratase-dehydrogenase-epimerase (HDE), peroxisomal 18-kDa protein (POX18), 2,4-dienoyl-CoA reductase (SPS19), acyl-CoA oxidase 4 (POX4), dienoyl CoA Isomerase (DCI) and acyl-CoA oxidase (POX5).”**

140. Claim 3 depends from Claim 1, which is disclosed by Bailey in view of Zhang as described above in ¶¶ 77-131. Similarly, Claim 3 is also rendered obvious by Bailey.

141. Claim 3 includes a list of specific fatty acid-inducible promoters used to induce expression of a terpene biosynthesis polypeptide. As described above in ¶¶ 106-107, Bailey describes POX4 and HDE as useful promoters, with Bailey

specifically citing to Zhang with respect to the POX4 promoter. Zhang, in turn, further describes POX4 and HDE as fatty acid-inducible promoters that can be used to express various genes, including those encoding for terpene biosynthesis polypeptides. Further, in p. 36 [00108], Bailey specifically recites 2 of the 6 promoters claimed in Claim 3 as examples of promoters that can be used to express a polypeptide. Specifically, Bailey describes hydratase-dehydrogenase epimerase(HDE) and acyl-CoA oxidase 4 (POX4) as promoters that provide for expression of polypeptides. Ex. 1003, Bailey at p. 36 [00108]. The discussion in ¶¶ 56 and 87 describes how these polypeptides include carotenoid biosynthesis polypeptides (i.e., terpene biosynthesis polypeptides) involved in the synthesis of carotenoids (i.e., terpenes).

142. Therefore, Bailey teaches or suggest each and every feature of dependent Claim 3 and renders the claim obvious.

B. Claims 1-3 are unpatentable under 35 U.S.C. §112(a) as lacking enablement in view of the specification

1. The breadth of the claims of the '148 Patent encompasses over 24 million possible permutations.

143. As discussed in ¶ 86 above, terpenes are a broad class of molecules composed of isoprene units (C₅H₈) “that can be joined together in a variety of different combinations to generate thousands of terpene compounds.” Ex. 1001, '148 Patent at p. 89 (58:31-35). In fact, the scientific field recognized that there were over

55,000 different terpene compounds identified by the priority date of the '148 Patent.
See Ex 1009, Maimone 2007 at p.1.

144. As discussed in ¶¶ 93-94, enzymes in the terpene biosynthesis pathway are “promiscuous” meaning that these enzymes can opportunistically convert various isoprene-based molecule substrates and produce an astoundingly diverse and complex set of different downstream products. Given this “biosynthetic promiscuity” of terpene biosynthesis pathway enzymes, the system claimed in the '148 Patent (even with being restricted to only the twelve enzyme classes named in Claim element 1[c] (*see* ¶¶ 117-120)) still has the potential to yield thousands of different terpene compounds. This diversity is further amplified by the chemical reactivities of intermediates in this pathway. Specifically, “intramolecular electrophilic attack of the intermediate allylic cations on the central or distal double bonds of the substrate....can account for the formation of the enormous variety of several hundred cyclic terpene compounds.” Ex. 1048, Yamada 2012 at p. 2. As a result, this terpene pathway is not a traditional, linear pathway that takes singular substrates and produces singular products.

145. As discussed in ¶¶ 143-144 above, and shown in Table 2A, a POSITA would understand that the breadth of Claim 1 would encompass genetically modified yeasts capable of generating >55,000 distinct terpene products.

TABLE 2A

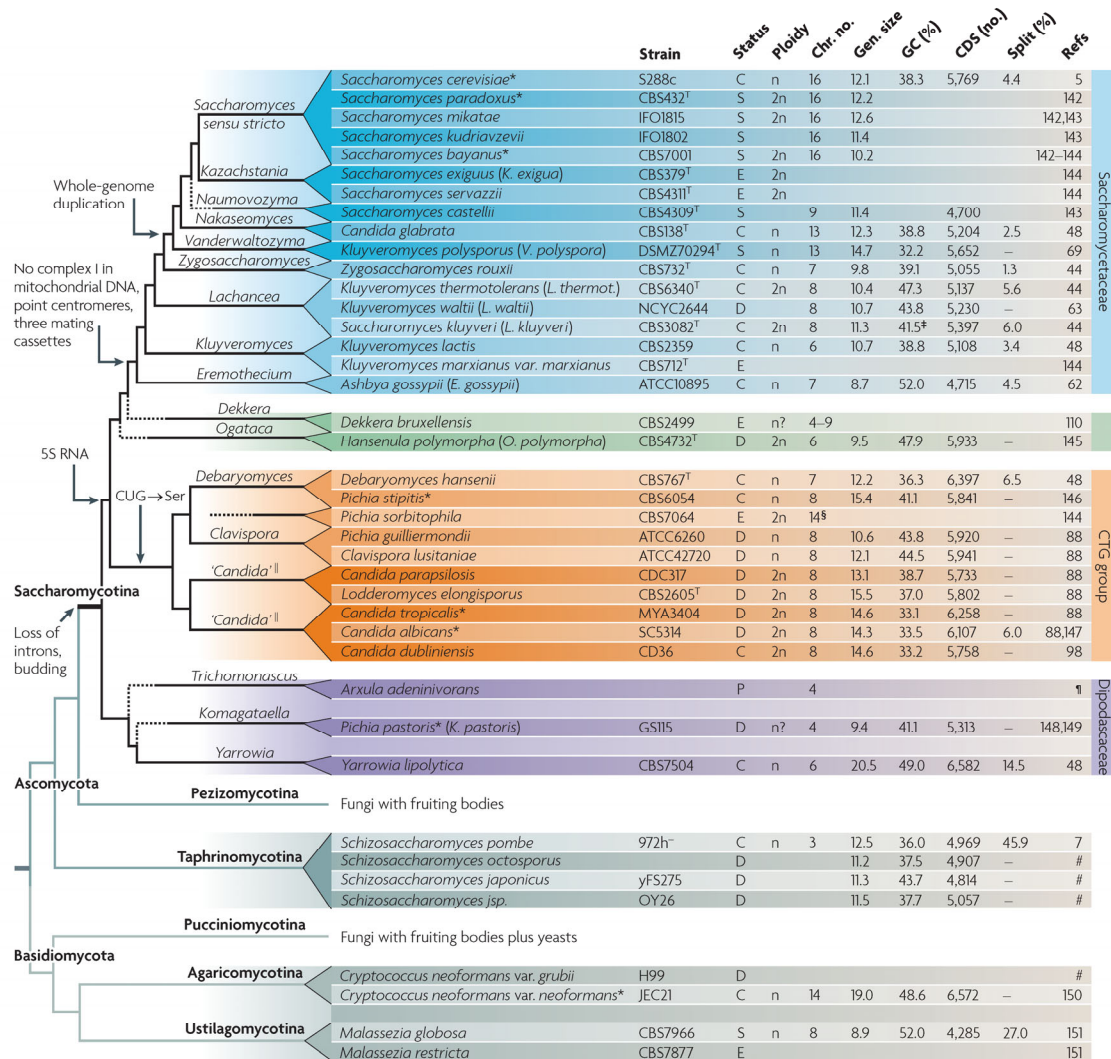
Claimed Group	Claimed Individuals
Terpene	>55,000 molecules

146. Moreover, Claim 1 encompasses much more than just a single organism having as few as one and as many as twelve distinct nucleotide sequences encoding up to twelve terpene biosynthesis enzymes. Rather, Claim 1 allows for such genetic combinations across two entirely distinct genera of yeast, *Candida* and *Blastobotrys*, encompassing a total of over 450 species. Ex. 1001, '148 Patent at p. 290 (Claim 1); ¶¶ 147-164.

147. Specifically, Claim 1 states that “the genetically modified microorganism is a yeast selected from the group consisting of *Candida* ssp [sic] and *Blastobotrys* ssp. [sic].”

148. A POSITA would understand that *Candida* and *Blastobotrys* are not only two separate genera but belong to two separate families of yeast. Genus *Candida* belongs to the family Saccharomycetaceae and genus *Blastobotrys* belongs to the family Trichomonascaceae. See Ex. 1030, Smith 2011; Ex. 1029, Lachance 2011. In particular, the *Blastobotrys* species clearly belongs to a small (and distinct) clade that is distinct from all other *Candida* species. See Ex. 1046, Suh 2006 at p. 9. The differences between *Candida* (with many species belonging to the CTG group—

a unique grouping in which the standard CUG codon encodes for serine instead of leucine) and *Blastobotrys* (all associated with the Dipodascaceae group) were known to the field and are quite evident in the following phylogenetic tree:



Ex. 1036, Dujon 2010 at p. 1 (Figure 1).

149. Despite this comparison, the genus *Candida* is not as homogenous as the rest in the phylogenetic tree above. Specifically, *Candida* is referred to as a “dumping ground” genus, as newly discovered species of yeast (based on crude

microscopic observations) are “dumped” into *Candida* and are subsequently frequently recategorized into different genera once further characterization is complete. *E.g.*, Ex. 1026, Kurtzman 2015 at p. 8. In this regard, “the *Candida* genus is poliphyletic and comprises species belonging to evolutionary distant clades....many *Candida* species are being renamed and the whole genus will likely be revamped in the coming years.” *See* Ex. 1037, Gabaldon 2016 at 2. Moreover, this classification method makes characterizing and comparing strains challenging. Put another way, “another continuing source of disparity is the use of the genus *Candida* as a dumping ground for most budding yeasts that do not form ascospores. Members of the current genus are found in essentially all teleomorphic clades.” *See* Ex. 1046, Suh 2006 at pp. 5-6.

150. A POSITA would understand that the species lumped into the genus *Candida* are generally categorized as such by the absence of a sexual form and consists of a diverse pool of species grouped into various clades that may be better suited as an individual genus than under the umbrella of *Candida*. *See* Ex. 1032, Barns 1991; Ex. 1039, Kidd 2023. Barns et al. describes “attempts . . . to define natural relationships among *Candida* species” as having been generally confounded by the great heterogeneity within the group which is further exacerbated by a previously used convention of assigning most species to “at least two genera (by

sexual and nonsexual forms),” (Ex. 1032, Barns 1991 at p. 1) a practice that has been abandoned since 2013 (Ex. 1039, Kidd 2023 at p. 1).

151. A POSITA would also recognize that two strains both classified as *Candida* could be highly disparate from one another. This is highly relevant as many *Candida* strains are medically relevant as they are human pathogens. A POSITA would be well-aware of this disparate classification throughout literature including warnings such as “For example, non-albicans *Candida* species comprise a random mixture of species that have been grouped together only because they are morphologically indistinguishable and physiologically similar, but some of them are as distant from each other as humans are from frogs. Applying names that acknowledge this diversity would be logical and medically meaningful.” (*emphasis added*) Ex. 1027, de Hoog 2015 at p. 2.

152. Further to this point, a study by Barns et al. of rRNA sequences of representative species categorized as *Candida* in 1991 found that *C. viswanathii* and *C. tropicalis*, both identified by Kidd et al. as members of the *Lodderomyces* clade, are quite similar, showing a pairwise sequence difference of 2 and an evolutionary distance of 0.16 in an experiment comparing 1240 positions. Ex. 1033, Barns 1991 at p. 1 (Table 3; Table 3, *infra*).

153. As seen in the reproduction of Table 3 of Barns 1991 below (*color emphasis added*), Barns et al. conducted two experiments measuring the genetic

difference between the rRNA of yeast species. The results of experiment 1 with respect to certain species have been highlighted in the reproduction of Table 3 below to aid in review of the data. The closely related species *C. viswanathii* and *C. tropicalis* are highlighted in blue. Certain of the other species identified as *Candida* species in 1991 that are specifically listed in the '148 Patent as exemplary *Candida* species are highlighted in green. Certain of the species mentioned but not claimed in the '148 Patent are highlighted in red. The intersection of columns and rows representing the species shows data regarding the genetic difference between the two intersecting species, as discussed further in ¶ 152 above and ¶¶ 154-155 below.

TABLE 3. Total pairwise sequence differences and evolutionary distances among fungal sequences examined

Expt and species	Pairwise sequence difference (lightface type) or evolutionary distance (boldface type), avg no. of changes per nucleotide × 100													
	<i>C. albicans</i>	<i>C. viswanathii</i>	<i>C. tropicalis</i>	<i>C. parapsi- losis</i>	<i>C. guillier- mondii</i>	<i>C. lusitanae</i>	<i>C. krusei</i>	<i>H. poly- morpha</i>	<i>T. glabrata</i>	<i>S. cere- visiae</i>	<i>C. kefyr</i>	<i>K. marxianus</i> var. <i>lactis</i>	<i>Y. lipo- lytica</i>	<i>A. fumi- gatus</i>
Expt 1 ^a														
<i>C. albicans</i>		4	4	6	14	49	86	46	38	55	47	47	117	107
<i>C. viswanathii</i>	0.32		2	2	14	50	82	44	37	52	46	47	117	109
<i>C. tropicalis</i>	0.32	0.16		2	12	50	82	43	36	52	44	46	117	110
<i>C. parapsilosis</i>	0.49	0.24	0.16		12	49	83	43	36	52	44	46	116	109
<i>C. guilliermondii</i>	1.10	1.02	0.93	0.93		48	84	38	36	48	38	42	111	104
<i>C. lusitanae</i>	3.96	4.05	3.97	3.96	3.75		97	70	63	77	65	68	131	112
<i>C. krusei</i>	6.86	6.51	6.61	6.59	6.55	7.86		71	79	90	80	81	158	146
<i>H. polymorpha</i>	3.61	3.45	3.36	3.36	2.98	5.68	5.52		36	47	42	42	119	113
<i>T. glabrata</i>	3.09	3.01	2.93	2.92	2.80	5.05	6.11	2.81		17	18	21	122	110
<i>S. cerevisiae</i>	4.23	4.15	4.07	4.06	3.75	6.13	7.12	3.69	1.34		27	28	132	121
<i>C. kefyr</i>	3.78	3.70	3.61	3.61	3.06	5.32	6.40	3.33	1.39	2.01		2	123	111
<i>K. marxianus</i> var. <i>lactis</i>	3.85	3.77	3.69	3.68	3.38	5.57	6.55	3.40	1.55	2.17	0.24		124	111
<i>Y. lipolytica</i>	9.86	9.78	9.79	9.77	9.35	11.02	13.66	9.96	10.20	11.07	10.32	10.57		156
<i>A. fumigatus</i>	8.96	9.05	9.15	9.14	8.63	9.34	12.29	9.51	9.10	9.96	99.21	9.28	13.37	
Expt 2 ^b														
<i>C. albicans</i>		11	11	16										
<i>C. viswanathii</i>	0.60		8	11										
<i>C. tropicalis</i>	0.70	0.41		13										
<i>C. parapsilosis</i>	0.95	0.67	0.76											
<i>T. glabrata</i>										41	55	58		
<i>S. cerevisiae</i>											55	59		
<i>C. kefyr</i>										3.31	3.18	5		
<i>K. marxianus</i> var. <i>lactis</i>									3.51	3.37	0.31			

^a Values were obtained using about 1240 unambiguous positions, constrained by the inclusion of the evolutionarily divergent *Y. lipolytica* sequence.

^b Values obtained using approximately 1600 positions among the organisms listed.

Ex. 1033 Barns 1991 at p. 1 (Table 3) (*color emphasis added*).

154. Despite the similarity between the specific species of *C. viswanathii* and *C. tropicalis*, Barns et al. also showed that there is a great deal of variability among species that have, at various points, been categorized as *Candida* species. Ex. 1033,

Barns 1991 at p. 1 (Table 3 *infra*). For example, the same rRNA experiment in Barns et al. found that *C. viswanathii* showed greater similarity to *Torulopsis glabrata* (now known as *Nakaseomyces glabrata*, but also formerly known as *Candida glabrata*) and *Hansenula polymorpha* (now known as *Ogataea polymorpha*, but also formerly known as *Candida thermophila*) than to *C. kefir* (now known as *Kluyveromyces marxianus*), *C. lusitaniae* (now known as *Clavispora lusitaniae*), or *C. krusei* (now known as *Pichia kudriavzevii*). *Id.* Likewise, *S. cerevisiae* shows greater similarity to *C. viswanathii* than *C. krusei* (now known as *Pichia kudriavzevii*) as well. *Id.* This comparison differs drastically from well-defined genus and clades whereby similarly groups species are similar. As de Hoog suggested, classifications based on morphology and physiology alone are not sufficient for identifying true genetic / phylogenetic distance. Ex. 1027, de Hoog 2015.

155. The information in Table 3, experiment 1 of Barns 1991 with respect to the highlighted species has been reorganized below as Table 3 for ease of reference. Because all of the working examples of fatty acid- or alkane-induced terpene production in the '148 Patent was carried out using *C. viswanathii*, Table 3 uses *C. viswanathii* as the reference species. The exemplary species highlighted and discussed in ¶¶ 152 and 154 and the reproduction of Table 3 of Barns 1991 above are arranged from most to least genetically similar to *C. viswanathii*. The column to

the left indicates whether or not the species is claimed in the '148 Patent. Table 3 also shows the sequence difference and the evolutionary distance between *C. viswanathii* and the species listed in the second column. The fewer sequence differences, the more closely related two species are; the greater the number of sequence differences, the more divergent two species are. Evolutionary distance represents the average number of sequence-based differences per nucleotide multiplied by 100 and was computed using pairwise analysis between strains. An evolutionary distance close to 0 indicates a close genetic relationship, while higher numbers indicate a more divergent genetic relationship. As discussed in ¶ 154, at least some of the species claimed as *Candida* species by the '148 Patent are more genetically divergent from the one working example in the '148 Patent than a non-*Candida* species.

TABLE 3

Claimed in '148 Patent	Species (arranged most to least related)	Sequence difference from <i>Candida</i> <i>viswanathii</i>	Evolutionary distance from <i>Candida</i> <i>viswanathii</i>
Yes	<i>Candida tropicalis</i>	2	0.16
Yes	<i>Torulopsis glabrata</i> (now <i>Nakaseomyces glabrata</i>)	37	3.01
-	<i>Hansenula polymorpha</i> (now <i>Ogataea polymorpha</i>)	44	3.45
Yes	<i>Candida kefir</i> (now <i>Kluyveromyces marxianus</i>)	46	3.70
Yes	<i>Candida lusitaniae</i> (now <i>Clavispora lusitaniae</i>)	50	4.05

Claimed in '148 Patent	Species (arranged most to least related)	Sequence difference from <i>Candida</i> <i>viswanathii</i>	Evolutionary distance from <i>Candida</i> <i>viswanathii</i>
-	<i>Saccharomyces cerevisiae</i>	52	4.15
Yes	<i>Candida krusei</i> (now <i>Pichia kudriavzevii</i>)	82	6.86

156. This pairwise comparison of distance aside, a POSITA would recognize the “dumping ground” nature of *Candida* strains and recognize that initial classification to the genus is not reflective of phylogenetic and genetic identity. As a result, strains are frequently (and properly) reclassified out of the *Candida* genus and into their correct genus (within which, phylogenetic and genetic similarities are more accurate and representative). As discussed in ¶ 157 and as illustrated on p. 181 (Table 4), the '148 Patent incorrectly identifies a number of species as “Candida” despite their having been reclassified to other genera prior to the priority date of the '148 Patent. *See* Ex. 1001, '148 Patent at p. 181 (Table 4). Thus, the '148 Patent incorrectly lists certain organisms as “suitable *Candida* species” such as to seemingly fall within the scope of the claimed embodiments, despite the fact that a POSITA would not have considered those organisms as *Candida* species by the priority date of the '148 Patent. Ex. 1001, '148 Patent at p. 93 (66:39-55); p. 181 (Table 4).

TABLE 4

Species identified in the '148 Patent as “suitable <i>Candida</i> species”	Classification as of July 13, 2017	Reclassification Date and Cite
<i>Candida famata</i>	<i>Debaryomyces hansenii</i>	Ex. 1040, Kreger-van Rij 1984 at p. 152.
<i>Candida glabrata</i>	<i>Nakaseomyces glabrata</i>	Ex. 1041, Kurtzman 2003 at p. 4.
<i>Candida guilliermondii</i>	<i>Meyerozyma guilliermondii</i> and <i>Pichia guilliermondii</i>	Ex. 1040, Kreger-van Rij 1984 at 692. P. 710
<i>Candida kefyr</i>	<i>Kluyveromyces marxianus</i>	Ex. 1040, Kreger-van Rij 1984 at p. 739.
<i>Candida krusei</i>	<i>Pichia kudriavzevii</i> and <i>Issatchenkia orientalis</i>	Ex. 1040, Kreger-van Rij 1984 at p. 744.
<i>Candida lipolytica</i>	<i>Yarrowia lipolytica</i>	Ex. 1019, van der Walt 1980 at p. 1.
<i>Candida lusitanae</i> [sic]	<i>Clavispora lusitanae</i>	Ex. 1040, Kreger-van Rij 1984 at p. 138 and p. 749.
<i>Candida rugosa</i>	<i>Diutina rugosa</i>	Ex. 1038, Khunnamwong 2015 at p. 1.

'148 Patent at p. 93 (66:39-55).

157. As a result, of the 18 species listed in 66:39-48 of the '148 Patent as “suitable *Candida* species” (Ex. 1001, '148 Patent at p. 93 (66:40-41)), 8 of these 18 were not actually *Candida*. In some cases, these strains were reclassified >35 years before the priority date of the '148 Patent. It is worth noting that although *C. glabrata* was proposed for reclassification to *Nakaseomyces glabrata* in 2003, full adoption of this rename was not completely recognized within the community until 2022 (after the priority date of the '148 Patent). See Ex. 1047, Takashima 2022. The challenges here are nonetheless illustrative of the ambiguous and improper classification of the *Candida* genus as well as the divergent genotypes within this species.

158. This mis-characterization of organisms within the '148 Patent is not without consequence. Specifically, as shown in Table 4, more than 35 years prior to the priority date of the '148 Patent, *C. lipolytica* was reclassified as *Y. lipolytica*. See Ex. 1019, van der Walt 1980. However, despite this, the '148 Patent lists *Candida lipolytica* as a “suitable *Candida* species.” Ex. 1001, '148 Patent at p. 93 (66:44; Table 4 *supra*).

159. Yet, the '148 Patent directly acknowledges the reclassification of *C. lipolytica* to *Y. lipolytica*, as evidenced by multiple references to “*Y. lipolytica* (formerly classified as *Candida lipolytica*).” Ex. 1001, '148 Patent at p. 93 (65:18-19, 63-65).

160. However, despite this direct acknowledgement of the reclassification, during the prosecution of the '148 Patent, the patentee allegedly narrowed the claimed yeast genera in part by cancelling the claim to the genus *Yarrowia*, but without making any reference to those contradictory and overlapping references within the patent specification. See Ex. 1002, '148 PH at 3025 and 3027.

161. The inclusion of multiple species as *Candida* that are no longer classified as *Candida* also introduces the idea that the '148 Patent is claiming any species that has ever been deemed *Candida*. As discussed above, given that *Candida* is referred to as a “dumping ground” and that a large number of diverse, genetically

divergent species have been classified as *Candida* at some point, this significantly increases the breadth of the '148 Patent's claims.

162. *Candida* is a broad genus containing species with a wide range of phenotypes and genetic diversity. A POSITA would have recognized that the classification is also quickly evolving and transitory as strains are reclassified. As an example of this, in 2011 (thus well understood by a POSITA by the priority date), there were 314 recognized species of *Candida* with an additional 51 being added during publication of a taxonomy anthology. See Ex. 1029, Lachance 2011. This number continued to increase by 2014 with 434 species being recognized as part of the *Candida* genus. See Ex. 1028, Daniel 2014. Thus, it would have been understood by a POSITA at the priority date of the '148 Patent that the *Candida* genus encompassed at least 430 distinct yeast species. Thus, claiming of the entirety of the *Candida* genus would be a broad claim in its own right and undue experimentation would be required to create additional embodiments of the '148 Patent in all the *Candida* species due to the inherent phenotypic and genetic differences. This point is discussed later in ¶¶ 175-180 wherein genetic modifications and heterologous expression in one *Candida* strain can fail to work in another species of *Candida*. The '148 Patent's specification, however, further broadens this claim by identifying as *Candida* an even broader swath of species including those that are no longer categorized as *Candida* due to considerable genetic divergence.

163. As discussed above in ¶¶ 124-126 and 146-148, Claim 1 further encompasses all of the organisms in the genus *Blastobotrys*. *Blastobotrys*, albeit a much smaller genus than *Candida*, would have been recognized by a POSITA to contain at least 20 distinct species by the priority date of the '148 Patent. See Ex. 1030, Smith 2011.

164. As discussed in ¶¶ 146-163 and shown in Table 2B, a POSITA would thus understand that the breadth of Claim 1 encompasses genetically modified yeasts across over 450 distinct species. Moreover, a POSITA would have recognized that many of these species are genetically and phylogenetically divergent from one another despite their initial taxonomy as *Candida*.

TABLE 2B

Claimed Group	Claimed Individuals
genus <i>Candida</i>	>430 species
genus <i>Blastobotrys</i>	>20 species

165. Moreover, in his October 20, 2022 declaration, Dr. Beardslee explicitly stated “which in the present Application is a specific genetically engineered microorganism, while in the prior arts cited are either generic oleaginous yeasts structurally different or promoters suitable for regulating the overexpression of beta-galactosidase in the yeast *Yarrowia lipolytica*”. Ex. 1002, '148 PH at p. 2944 (¶ 19). While I agree with Dr. Beardslee’s opinion that only *specific* microorganisms should

be considered, this declaration statement is in direct opposition to the reality of selecting over 450 unrelated, non-specific microorganism choices.

166. When viewed in its entirety, the combination of the breadth of terpene products claimed (> 55,000) together with the breadth of species claimed (> 450) further magnifies the possible permutations encompassed under Claim 1 of the '148 Patent. As illustrated in Table 2C below, with 450 species and at least 55,000 terpene molecules, the '148 Patent claims at least 24,750,000 possible permutations simply based on the pathway and species alone. This number does not account for other variables encompassed by the '148 claims (*e.g.*, induction by different fatty acids or alkanes; the use of different promoters). The addition of dependent Claim 3 simply amplifies this number six-fold (when considering the inclusion of the six promoter possibilities of Claim 3) to 148,500,000 possible permutations.

TABLE 2C: CLAIMED PERMUTATIONS

Claimed Group	Claimed Individuals
genus <i>Candida</i>	>430 species
genus <i>Blastobotrys</i>	>20 species
Terpene	>55,000 molecules
<i>Candida</i> or <i>Blastobotrys</i> x Terpene	>24,750,000 permutations

2. In claiming over 24 million permutations, the '148 Patent overlooks the inherent complexity of expressing heterologous biosynthesis pathways and thus lack of sufficient predictability of the art.

167. It is well-known in the art that genetic modification of an organism can be used to enable the expression of a heterologous gene of interest; in short, genetic modification can make an organism generate a product that it ordinarily would not produce. *E.g.*, Ex. 1008, Alberts 2008 at p. 158 (Chapter 8). At a simple level, a gene encoding a specific polypeptide can be introduced into the organism's genome, a promoter (either constitutive, i.e., always on or inducible, i.e., conditionally on) can be used to facilitate transcription of mRNA transcripts, and the mRNA transcripts may then be translated into amino acid chains called polypeptides. *E.g.*, *id.* at 158. The resulting polypeptide must undergo further processing, including any appropriate post-translational modification steps and folding for the polypeptide to form a fully functional protein. *E.g.*, *id.* Post-translational modification and folding depend on a number of factors including pH within a cell and compatibility or expression of native enzymes that promote post-translational modification. *E.g.*, *id.* at p. 76 (Chapter 6) and especially at p. 135. Inadequate post-translational modification or misfolding may result in a non-functional or sub-functional protein. *E.g.*, *id.* at p. 76 (Chapter 6) and especially at pp. 237-240.

168. A protein that catalyzes a chemical reaction is called an enzyme (examples include geranylgeranyl diphosphate synthase and lycopene cyclase). *See*

Ex. 1008, Alberts 2008 at p.7 (Chapter 3) and especially at 40. Enzymes must be able to interact with a substrate (such as the isoprene-based precursor molecules isopentenyl diphosphate and farnesyl pyrophosphate) to catalyze such reactions. *E.g., id.* at p. 7 (Chapter 3) and especially at 40-41. A biosynthesis pathway relies on the interaction of multiple proteins, including enzymes, with one another and/or with a specific substrate to synthesize a product (*e.g.*, β -carotene and lycopene). *E.g., id.* at p. 7 (Chapter 3) and especially at 50-52.

169. A POSITA would recognize that this means that, in order to introduce a non-native biosynthesis pathway into an organism, the process described in ¶ 167 must be repeated for each protein along the biosynthesis pathway. As described in further detail below in ¶¶ 170-172 and 181-186, a POSITA would recognize that any inadequacies (*e.g.*, expression errors, misfoldings, incompatibility, improper cellular localization) along the way are multiplied by the need for each protein to function adequately along the pathway—if two of three proteins along the pathway are perfectly folded and modified but the third is not, the biosynthesis pathway likely will not synthesize the target product. If all three of the proteins are perfectly folded and modified but cannot interact properly with other proteins and/or with a substrate (*e.g.*, due to pH, competitive interference with or chelation of a substrate, missing cofactors, aggregation in the cell, etc.), the biosynthesis pathway might not synthesize the target product. These conditions notwithstanding, the proper

expression of multiple enzymes could still be subject to alternative forms of allosteric regulation and/or feedback inhibition that would render the pathway nonfunctional. *E.g.*, Ex. 1008, Alberts 2008 at p. 7 (Chapter 3).

170. Adding further complexity, as discussed in ¶¶ 93-94, the enzymes in a biosynthesis pathway may be “promiscuous” – interacting with multiple substrates to yield multiple products. If an enzyme encounters substrate A, it may yield product Y, but if the same enzyme encounters substrate B, it may yield product Z. Therefore, if product Y is the target product, it is not sufficient to merely express fully functioning enzymes, but rather, the system must be manipulated such as to promote production of product Y over product Z. This overall principle forms the central premise for the field of Metabolic Engineering. *E.g.*, Ex. 1031, Bailey 1991.

171. Moreover, an already complex system may be made even more complex through the substitution of constitutive promoters with inducible promoters, as discussed in further detail in ¶ 182 and in ¶ 209-210 below.

172. A relatively simple gene expression system producing a single polypeptide may be relatively predictable and might be enabled by a few working examples, especially for cases wherein very similar species are selected. A POSITA would recognize, however, that as the complexity of the system increases (either through more pathway enzymes or more diverse species), so too does the unpredictability and the need for more working examples. The modified yeast

claimed in the '148 Patent biosynthesizes terpenes as a downstream product of expressing terpene biosynthesis pathway proteins in the presence of fatty acids or alkanes. This is a particularly broad claim given that, by the Patent Owner's own admission, "[a]s an initial matter, even just the choice of a suitable promoter requires an extensive trial and error and a deep understanding of the processes and the parameters to be checked and selected." Ex. 1002, '148 PH at p. 2960. As a POSITA, I concur with the assessment that the choice of a promoter does require trial and error and a deep understanding of the processes and parameters to be considered.

173. In his October 20, 2022 declaration, Dr. Beardslee explicitly stated, "I declare that the specific choice of a promoter for any biological system will surely require an extensive trial and error and a deep understanding of the processes and the parameters to be checked and selected." Ex. 1002, '148 PH at p. 2944 (¶ 19). As a POSITA, I concur with the assessment that the choice of a promoter does require trial and error and a deep understanding of the processes and parameters to be considered.

174. In fact, Dr. Beardslee stated that the promoters suggested by a prior art reference, Juretzek, which was being cited as evidence of obviousness, could not be guaranteed to function in a different host. Specifically, he points to these promoters as being:

selected for a specific biotechnological system, namely Yarrowia on oleic acid. However, in the case of different systems, such as for

example a Candida viswanathii system, these promoters (POX2, POT1, and ICL1) showed in the mRNA sequencing data very poor overall expression levels on oleic acid. Hence, even if the person skilled in the art reading Juretzek would have taken into account the teachings contained thereof, he/she would have chosen poorly working promoters, hence he/she would have never reached the solution for the technical problem above described.

Ex. 1002, '148 PH at p. 2946 (¶ 28).

175. As a POSITA, I recognize that Dr. Beardslee's description of poor and failed testing of the promoter from *Y. lipolytica* (a species formerly known as *C. lipolytica* and referenced as such by the '148 Patent as described above in ¶¶ 110, 123 and 159, and as referenced throughout the '148 Patent as a "suitable Candida species" (Ex. 1001, '148 Patent at p. 93 (66:40-41)) in *C. viswanathii* shows that the examples of fatty acid- and/or alkane-inducible promoters provided in the '148 Patent's specification are not necessarily transferable to other microorganisms.

176. Moreover, the literature pre-dating the priority date of the '148 Patent also supports this understanding in Dr. Beardslee's Declaration as well as my opinions. Specifically, not only is the notion of transferability of promoters across the *Candida* genus not guaranteed, but it is also explicitly debunked in the literature in a manner that would have been well-known to a POSITA. A common understanding in the field was that advances in genome sequencing tools and databases were helping to "facilitate the isolation of homologous constitutive or regulatable promoters and terminators for use in specific *Candida* species."

(*emphasis added*) Ex. 1043, Papon 2012 at p. 13. Specifically, a POSITA would have had an understanding that genetic tools would be specific to individual *Candida* species and not universally applicable. Put another way, transcriptional regulators (like inducible promoters) that work in one strain will not work in another.

177. A further example of this is limitation is evident in work that attempted to utilize a well-characterized ACT1 promoter from *C. albicans* as a heterologous nucleic acid in the strain *C. guilliermondii*. Ex. 1042, Millerioux 2011. It should be noted that both of these species are “suitable *Candida* species” according to the ’148 Patent. Ex. 1001, ’148 Patent at p. 93 (66:40-41). However, the Millerioux 2011 study failed to achieve any results using this construct. The researchers had to instead design new endogenous elements from *C. guilliermondii* that formed the basis of an entire research paper just to establish tools in this strain. Ex. 1042, Millerioux 2011. The authors rightfully concluded that “[i]t is thus likely that these heterologous transcription-regulating sequences preclude the expression of SAT-1 and HPH# in *C. guilliermondii*.” *Id.* at p. 4. This basic understanding that heterologous nucleic acids, especially promoters, can fail to function across species is also reflected in Dr. Beardslee’s Declaration.

178. Furthermore, a POSITA would unequivocally understand the challenges of transferring genetics in a loosely associated genus such as *Candida*.

Such a point is highlighted when comparing *C. albicans* to non-*C. albicans* (NAC) species (all species that are included in the *Candida* genus at present). Specifically:

Due to the lack of genetic and molecular resources, researchers have often assumed that if a yeast species is related to another yeast species, the underlying molecular and cellular mechanisms must also be closely related. However, even within a clade, the genetic distance between any two NAC species is often larger than the genetic distance between humans and some fishes. Therefore, in no way should it be argued that C. albicans makes the rules for all NAC species. As a corollary, in future investigations, the biology of each Candida species should continue to be addressed on a case-by-case basis.

Ex. 1044, Papon 2013 at p. 3. (*emphasis added*)

179. As a result, a POSITA would understand that studying the biology of each species “on a case-by-case basis” would require extensive experimentation and learnings from one species would not guarantee success in the next species.

180. Unlike in the obviousness discussion of Zhang in ¶¶ 107-116 above where the POX4 promoter is explicitly called out as strongly induced in multiple yeast strains, the ’148 Patent merely lists POX2, POT1, and ICL1 as examples of *Y. lipolytica* (formerly known as *C. lipolytica*) promoters without qualification as to their functionality. Furthermore, these promoters are, by Dr. Beardslee’s own admission, not interchangeable with *C. viswanathii* and thus would not work in the *C. viswanathii* system and would fail to achieve the reported invention of the ’148

Patent. By extension, a POSITA would understand this to mean that the examples of promoters listed in the specification are only instructive with respect to the specific microorganism for which it is listed and that identification of any promoter not explicitly listed in the specification in association with a specific microorganism and a specific inducer would require—in the words of Dr. Beardslee—“extensive trial and error.” Ex. 1002, ’148 PH at p. 2944 (¶ 19).

181. Moreover, a POSITA would understand that establishing a non-native biological pathway with the hope of producing a downstream product is inherently complex with multiple inflection points. As discussed above in ¶ 172-180, the initial hurdle for introduction of a non-native pathway to a microorganism lies in creating a genetic construct to express the proteins, which has its own obstacles and inherent unpredictability, particularly with applicability across species. *E.g.*, Ex. 1049, Zhang 2010. However, the biosynthesis of terpenes via heterologous expression of a pathway does not end with gene expression and synthesis of a single non-native protein but instead is a rather complex process involving numerous other molecules and strain engineering strategies. *See, e.g.*, Ex. 1045, Paramasivan 2017, discussing the state of the art in a model yeast organism *S. cerevisiae*. Specifically, it is stated: “The underlining theme for successful production of a terpene discussed here was possible due to the combination of several metabolic engineering strategies but not to an isolated strategy alone. Moreover, the success of commercial exploitation

depends on how these molecular strategies work in tandem with process development” *Id.* at p. 13. Thus, there are multiple steps beyond heterologous expression that are required to achieve terpene synthesis in a new fungal host.

182. In fact, Dr. Beardslee appears to agree and differentiates the complexity of introducing a non-native biosynthesis pathway from the general expression of a single gene product saying, “beta-galactosidase is a single gene product that is easily expressed in many microbiological systems as an enzyme with an easy activity assay.” Ex. 1002, ’148 PH at p. 2945 (¶ 24). Dr. Beardslee then contrasts this point by noting:

the necessity of the use of a promoter, its precise choice and its specific modifications, depend on several crucial parameters, such as: - the specific induction upon exposure to the nutrients (which only CAN be but surely not mandatorily be fatty acids), - the specific fermentation system used (which in the present Application is a specific genetically modified microorganism, while in the prior arts cited are either generic oleaginous yeasts structurally different or promoters suitable for regulating the overexpression of beta-galactosidase in the yeast *Yarrowia lipolytica*), - the biochemical pathways desired, and - the overall level of expression of the targeted genes.

Id. at ¶ 19.

183. I agree with Dr. Beardslee’s assessment that introduction of beta-galactosidase gene into a cell for the purpose of generating a single enzyme and then

testing for the functional presence of that enzyme is a well-known genetic modification, although not always a successful endeavor. I also agree with Beardslee's assessment that the introduction of non-native biosynthesis pathways is much more complex and unpredictable.

184. For example, in a system where non-native sequences encoding biosynthesis enzymes have been inserted into an organism with the intent of establishing a non-native biosynthesis pathway, it is not only sufficient that the desired polypeptides are synthesized. The polypeptides must also be expressed and folded properly into a protein product and the protein product must function within the terpene biosynthesis pathway. Synthesis of non-native proteins are particularly susceptible to misfolding and inadequate post-translational modification because the conditions inside the genetically modified organism may preclude proper folding and modification. Even more so, it the expression of each heterologous protein is often a one-off optimization specifically related to the protein of interest. *E.g.*, Ex. 1049, Zhang 2010 at p. 8. Even if synthesized and appropriately modified, the proteins may not interact as needed to produce terpenes. The unpredictability and inherent complexity of non-native proteins to function as expected is illustrated in Example 18 of the '148 Patent, wherein modified carnitine acetyltransferase constructs have differing and not entirely predictable abilities to restore growth on fatty acids to a Cat2 strain.

185. Furthermore, even if the desired proteins are synthesized and function in a manner necessary to synthesize terpenes, the proteins may not be produced at high enough levels to actually generate terpenes without more than a reasonable amount of experimentation. Achieving the correct balance of enzyme expression levels, especially for the case of enzymes with promiscuous activity, are required to flux-balance the metabolic pathway of interest and achieve bioproduction of the desired product. *See* Ex. 1031, Bailey 1991.

186. As discussed in ¶ 170 and explained in further detail in ¶¶ 93-94, the enzymes in a biosynthesis pathway may interact with multiple substrates to yield multiple products. This means that variables such as the level and rate of gene expression and the relative abundance of one substrate over another could impact whether a terpene is produced via a non-native terpene biosynthesis pathway, further adding unpredictability and complexity to the system.

3. Despite claiming over 24 million permutations in the highly unpredictable art space of complex biosynthesis pathway manipulations, the '148 Patent fails to articulate how additional embodiments could be reliably generated.

187. The '148 Patent purports to provide 75 examples of the invention but only seven examples show actual terpene production in response to fatty acid or alkane induction (i.e., the method of Claim 1). Ex. 1001, '148 Patent at p. 255 (Example 33), p. 256 (Example 37), p. 393 (Example 40), p. 258 (Example 46), p. 258 (Example 48), p. 259 (Example 54), and p. 260 (Example 61). Despite the

numerosity of the sections of the '148 Patent that ostensibly outline the means by which a future POSITA could reproduce and exercise the claimed material, such POSITA would understand that the amount of direction found in the examples of the '148 Patent are nothing more than a description of trial-and-error methods that the inventors encountered while developing the working embodiments rather than actual directions to allow a POSITA to create the claimed invention at its full breadth. For example, in Example 36 of the '148 Patent, the description of strain construction simply states that “[t]he TPS1 Gene from *Callitropsis nootkatesis* (CnTPS1) was codon optimized for *C. viswanathii*,” but the description fails to describe why the TPS1 gene from *C. nootkatesis* was chosen as opposed to the TPS1 gene from a different organism or how the gene was optimized for *C. viswanathii*. Ex. 1001, '148 Patent at p. 256 (391:45-67). Moreover, this example indicates the use of a “HDE1 promoter with a POX4 terminator” (Ex. 1001, '148 Patent at p. 256 (391:51-52)) without any mention as to the rationale for this selection. More worrisome, this combination of HDE1 promoter and POX4 terminator differs from the (again, unsubstantiated) Example 1, Table 3 (Ex. 1001, '148 Patent at p. 177 (Table 3)) construct whereby the HDE1 promoter is shown with the PEX11 terminator (notably, this is also the only entry in this table to have a promoter not placed alongside its cognate terminator without any justification). This unsubstantiated

difference once again highlights the “trial and error” methods rather than a concerted roadmap with logic and justification for a POSITA to follow.

188. In another instance, Example 29 merely recites a transformation protocol devoid of any data to demonstrate efficacy, efforts to demonstrate this across species, or any relevancy to showing an actual example of the reported invention.

189. In yet another example, multiple Examples (*e.g.*, 64 and 65 among many others) discuss the strain construction efforts in various mutant backgrounds of a singular strain. No effort is provided to explain the differences or limitations of these background strains. Nor are these examples relevant to expanding the demonstration of Claims 1-3 to other species of *Candida* or *Blastobotrys*. These efforts are merely aimed at alternative approaches that are ancillary to the claimed invention and, if nothing else, mimic the mutations and engineering efforts disclosed in Bailey instead.

190. Many additional “examples” throughout the ’148 Patent relate to methods of transformation, PCR, and cell culturing. Many of these can be viewed as standard methods of the field and not in any way a working example to exemplify the presently proposed invention. This said, many details provided here deviate (in an unjustified manner) from standardized media. For example, the use of 75 g/L glycerol (*see, e.g.*, Ex. 1001, ’148 Patent at p. 191 (262:46-47)) is rather high for

traditional yeast media which contains 20 g/L sugar content. There is no roadmap or justification here nor an explanation as to how this can be applied to other species of *Candida* or *Blastobotrys*.

191. Further, a POSITA would recognize that the specification provides little practical guidance for constructing a yeast capable of fatty acid- or alkane-induction of terpene production, particularly with respect to *Blastobotrys*. For example, as discussed in ¶ 182, Dr. Beardslee states that among the parameters a POSITA would need to consider and combine are specific induction upon exposure to nutrients, the fermentation system used, the biochemical pathways desired (which is not just the selection of the specific set of enzymes to express but, as discussed in ¶ 170, may require manipulation and timing of protein expression so as to overcome the promiscuity of terpene biosynthesis pathway enzymes) and overall gene expression. Ex. 1002, '148 PH at p. 2944 (¶ 19).

192. In fact, the instructive portions of the specification are devoid of any guidance for generating embodiments in *Blastobotrys* species. For example, while the specification lists some “[f]atty acid- and/or alkane-inducible promoters” from *S. cerevisiae*, *Yarrowia lipolytica*, *C. tropicalis*, and *C. albicans*, the specification fails to identify suitable promoters in *Blastobotrys*. As discussed above in ¶ 173, inventor Beardslee explicitly stated, “I declare that the specific choice of a promoter for any biological system will surely require an extensive trial and error and a deep

understanding of the processes and the parameters to be checked and selected.” Ex. 1002, ’148 PH at p. 2944 (¶ 19). As further discussed above in ¶ 174-175, by inventor Beardslee’s own admission, the three promoters provided as examples of *Y. lipolytica* (formerly known as *C. lipolytica*) promoters (POX2, POT1, and ICL1) are not interchangeable with *C. viswanathii* and would not work in *C. viswanathii*. Thus, the specification of the ’148 Patent provides no guidance on how a POSITA may select suitable promoters for *Blastobotrys* species nor do they provide any guarantees that any described promoter construct would function in *Blastobotrys*.

193. Moreover, there are no working examples of *Blastobotrys* in the ’148 Patent itself. Instead, a single working example in *Blastobotrys adeninivorans* was described in the March 22, 2023 Beardslee Declaration stating briefly “since oleic acid was the primary carbon source, all genes were expressed under a *Blastobotrys adeninivorans* POX promoter with a *Blastobotrys adeninivorans* POX terminator.” Ex. 1002, ’148 PH at p. 3032 (¶ 13). No additional guidance is provided that would allow a POSITA to generate an embodiment in other *Blastobotrys* species or using other promoters beyond POX. *Id.* More troublesome, this declaration does not provide sufficient details for a POSITA to follow and replicate even within the same species of *Blastobotrys* without undue and extensive trial-and-error. It was well understood at the time that *B. adeninivorans* was closely related to the strain *Y. lipolytica*. See Ex. 1023, Kunze 2014, especially at p. 16-17 (Figure S7A). It would

have been well known to a POSITA that in *Y. lipolytica*, the POX genes represent “a family of six peroxisomal acyl-coenzyme oxidases encoded by the POX1 to POX6 genes.” See Ex. 1034, Beopoulos 2008 at p. 2). Moreover, Dr. Beardslee clearly recognized multiple POX genes and in fact identified only 2 of which as suitable for inclusion in Example 1, Table 3 (specifically, POX4 and POX5). Ex. 1001, ’148 Patent at p. 177 (Table 3). In his March 22, 2023 Declaration, Dr. Beardslee’s only data for *Blastobotrys* only refers to “POX promoter” generically. Ex. 1002, ’148 PH at p. 3032 (¶ 13). As such, a POSITA would have been subjected to “extensive trial and error” as even this additional data lacks full support and justification (in a manner similar to the text of the specifications).

194. Further, while the working example states that various genes were “codon optimized for *B. adenivorans* and synthesized,” the example fails to provide any further direction as to how the genes were codon optimized and why genes from some organisms were used instead of others, or what combination of heterologous promoter/terminator systems were utilized, among other important details for a POSITA to achieve at this same invention. Ex. 1002, ’148 PH at p. 3032 (¶ 13).

195. Taken together, even with the addition of the working example of an embodiment in *Blastobotrys adenivorans*, within a declaration, there is no guidance as to how a POSITA should select the various elements, including

promoters, that would allow them to generate other working embodiments in *Blastobotrys* species with a reasonable likelihood of success. In short, in my opinion, the specification does not enable any embodiments in *Blastobotrys* and the single working embodiment described in the Beardslee Declaration only enables a single narrow embodiment: oleic acid-inducible production of lycopene in *Blastobotrys adeninivorans* through POX-regulated expression of terpene enzyme-encoding genes from three completely different organisms.

196. Finally, it should be noted that even Dr. Beardslee in his March 22, 2023 declaration recognized the significant limitations of his own research and additional data in declaring “I declare that the *Blastobotrys* yeast strain and the *Candida* yeast strains can both be considered microorganism strains that provide for terpene biosynthesis as presently claimed.” Ex. 1002, ’148 PH at p. 3033 (¶ 14). In singling out only “the *Blastobotrys* yeast strain” and not the entire genus as he does with *Candida*, this admission demonstrates the inability to extend the provided data to alternative species within the genus. I agree with this aspect of Dr. Beardslee’s admission in that this work cannot be extrapolated across other species within the genus without “extensive trial and error.” Together with this limitation and the issues described above, neither the specifications nor additional information in the declaration provides for a roadmap to extend to other species with any confidence.

197. Even in the genus *Candida*, the specification of the '148 Patent provides limited and insufficient guidance on how a POSITA could create embodiments in more distantly related species that the '148 Patent claims as purported *Candida* species or to create embodiments that produce different terpene products, particularly with respect to how the terpene pathway may be manipulated to regulate the production of various subproducts.

198. As discussed above, *Candida* is a very diverse genus and due to the unpredictability inherent to the genetic modification of genetically divergent species, more than a reasonable amount of experimentation would be required to generate additional embodiments in *Candida* species outside of *C. viswanathii*.

199. Furthermore, in light of the Dr. Beardslee's own admission, the illustration of a few working examples limited to three terpenes produced in one species of *Candida* and a single terpene produced in a single species of *Blastobotrys* is not sufficient to enable a POSITA to generate the breadth of the embodiments claimed without undue experimentation, particularly when Dr. Beardslee admits that the elements involved are not interchangeable without undue experimentation and when the specification fails to provide guidance as to how to adjust the parameters such as to achieve such additional embodiments.

200. A POSITA would understand that the '148 Patent specification provides little guidance as to how entire sets of terpene biosynthesis pathway

proteins may be chosen to produce terpenes. The working examples that describe the production of terpenes (lycopene, β -carotene, and valencene) fail to show the full range of terpenes being produced or how such a full range might be produced.

201. A POSITA would recognize that generating additional embodiments producing alternative terpenes would require more than a reasonable amount of experimentation, particularly because of the need to introduce a non-native biosynthesis pathway for the alternative terpene, which is a complex task, as described in further detail in ¶¶ 167-186 above.

4. **Despite claiming over 24 million permutations of microorganisms producing terpenes and extremely limited guidance on how to generate successful embodiments, the '148 Patent provides only 15 working examples – 13 of which are in the same *C. viswanathii* strain – and which illustrate synthesis of only 3 terpene products (lycopene, β -carotene, valencene).**

202. As discussed in ¶ 166 and illustrated in Table 2C (reproduced below for ease of reference), the '148 Patent claims over 24,750,000 permutations when accounting for selection microorganism and terpene alone but only provides examples of 4 such permutations.

TABLE 2C repeated

Claimed Group	Claimed Individuals	Exemplary Individuals
genus <i>Candida</i>	>430 species	1 species

genus <i>Blastobotrys</i>	>20 species	1 species (if including the material provided in the declaration)
Terpene	>55,000 molecules	3 molecules
<i>Candida</i> or <i>Blastobotrys</i> x Terpene	>24,750,000 permutations	4 permutations

203. Despite claiming all genetically modified yeasts in the genera *Candida* and *Blastobotrys* (genera that include hundreds of distinct species and strains with differing genetic sequences and levels) that are modified to express terpenes (a broad category of molecules that encompass tens of thousands of possible molecules) selectively in the presence of fatty acids or alkanes, the '148 Patent provides examples of terpene production in the presence of a fatty acid or alkane in only a single species of *Candida* and a single species of *Blastobotrys*. Moreover, as indicated in Table 5 below, despite the broad scope of the claims which encompass the capability of generating tens of thousands of terpenes, the '148 Patent provides examples only three terpenes being generated – lycopene, β - carotene, and valencene. Ex. 1001, '148 Patent at p. 255 (Example 32), p. 255 (Example 33), p. 256 (Example 39), p. 257 (Example 40), p. 258 (Examples 45-49), p. 259 (Example 52), p. 259 (Example 54), p. 259 (Example 57), p. 260 (Example 58), p. 260 (Example 60), p. 260 (Example 61), pp. 261-262 (Examples 67-69 (for lycopene)), p. 258 (Examples 47-48 (for β -carotene)), and p. 256 (Examples 36-37 (for valencene)); and Ex. 1002, '148 PH at p. 3033 (Table 1 (for lycopene)).

204. The specification of the '148 Patent shows fatty acid-induced terpene production in only one species of *Candida*, *C. viswanathii*, and a declaration filed in response to an Examiner's comment regarding the potential for lack of enablement provides a single working example for *Blastobotrys*, a *Blastobotrys adeninivorans* that produces lycopene.

205. As indicated in Table 5 below, the '148 Patent and its prosecution history provides limited working examples of fatty acid-induced terpene production. As mentioned above, the examples show fatty acid-induced lycopene production in only one species of *Candida* and one species of *Blastobotrys*. As indicated in Table 5 below, further permutations of the other claimed variables were carried out in a single *Candida* species. As noted above, embodiments use *C. viswanathii* to produce only three terpenes: lycopene, β -carotene, and valencene. The working examples illustrate the use of eight heterologous nucleic acids encoding five different terpene biosynthesis proteins from four species of organisms: archaea, bacteria, yeast, and trees. The working examples also only show use of three promoters.

Table 5

	Strain	Example No.	1[a] heterologous nucleic acids encoding	1[b]/1[d] promoter	1[c] terpene biosynthesis enzymes	1[e] <i>Candida</i> ssp or <i>Blastobotrys</i> ssp.	1[f] terpene product
1	sAA7446	32-33	<i>Cronobacter sakazakii</i> CsCrtE <i>Cronobacter sakazakii</i> CsCrtB <i>Cronobacter sakazakii</i> CsCrtI	HDE1	geranylgeranyl diphosphate (also known as pyrophosphate) synthase (CrtE) phytoene synthase (CrtB), phytoene desaturase (CrtI)	<i>Candida viswanthii</i>	lycopene
2	sAA7565	39-40	<i>Cronobacter sakazakii</i> CsCrtE <i>Cronobacter sakazakii</i> CsCrtB <i>Cronobacter sakazakii</i> CsCrtI	HDE1	geranylgeranyl diphosphate synthase (CrtE), phytoene synthase (CrtB), phytoene desaturase (CrtI)	<i>Candida viswanthii</i>	lycopene

	Strain	Example No.	1[a] heterologous nucleic acids encoding	1[b]/1[d] promoter	1[c] terpene biosynthesis enzymes	1[e] <i>Candida</i> ssp or <i>Blastobotrys</i> ssp.	1[f] terpene product
3	sAA8283	45, 46, 49	<i>Cronobacter sakazakii</i> CsCrtE <i>Cronobacter sakazakii</i> CsCrtB <i>Cronobacter sakazakii</i> CsCrtI	HDE1	geranylgeranyl diphosphate synthase (CrtE), phytoene synthase (CrtB), phytoene desaturase (CrtI)	<i>Candida viswanthii</i>	lycopene
4	sAA8519	47, 48	<i>Cronobacter sakazakii</i> CsCrtE <i>Xanthophyllomyces dendrorhous</i> XdCrtYB <i>Cronobacter sakazakii</i> CsCrtI	HDE1	geranylgeranyl diphosphate synthase (CrtE), bifunctional lycopene cyclase/phytoene synthase (CrtYB), phytoene desaturase (CrtI)	<i>Candida viswanthii</i>	Lycopene β-carotene

	Strain	Example No.	1[a] heterologous nucleic acids encoding	1[b]/1[d] promoter	1[c] terpene biosynthesis enzymes	1[e] <i>Candida</i> ssp or <i>Blastobotrys</i> ssp.	1[f] terpene product
5	sAA8932	52, 54	<i>Xanthophyllomyces dendrorhous</i> XdCrtE <i>Cronobacter sakazakii</i> CsCrtB <i>Xanthophyllomyces dendrorhous</i> XdCrtI	GPD	geranylgeranyl diphosphate synthase (CrtE), phytoene synthase (CrtB), phytoene desaturase (CrtI)	<i>Candida viswanthii</i>	lycopene
6	sAA9432	57, 58	<i>Xanthophyllomyces dendrorhous</i> XdCrtE <i>Cronobacter sakazakii</i> CsCrtB <i>Xanthophyllomyces dendrorhous</i> XdCrtI	HDE1	geranylgeranyl diphosphate synthase (CrtE), phytoene synthase (CrtB), phytoene desaturase (CrtI)	<i>Candida viswanthii</i>	lycopene

	Strain	Example No.	1[a] heterologous nucleic acids encoding	1[b]/1[d] promoter	1[c] terpene biosynthesis enzymes	1[e] <i>Candida</i> ssp or <i>Blastobotrys</i> ssp.	1[f] terpene product
7	sAA9670	60, 61	<i>Xanthophyllomyces dendrorhous</i> XdCrtE <i>Cronobacter sakazakii</i> CsCrtB <i>Xanthophyllomyces dendrorhous</i> XdCrtI	HDE1	geranylgeranyl diphosphate synthase (CrtE), phytoene synthase (CrtB), phytoene desaturase (CrtI)	<i>Candida viswanthii</i>	lycopene
8	sAA9811	67, 69	<i>Xanthophyllomyces dendrorhous</i> XdCrtE <i>Cronobacter sakazakii</i> CsCrtB <i>Xanthophyllomyces dendrorhous</i> XdCrtI	HDE1	geranylgeranyl diphosphate synthase (CrtE), phytoene synthase (CrtB), phytoene desaturase (CrtI)	<i>Candida viswanthii</i>	lycopene

	Strain	Example No.	1[a] heterologous nucleic acids encoding	1[b]/1[d] promoter	1[c] terpene biosynthesis enzymes	1[e] <i>Candida</i> ssp or <i>Blastobotrys</i> ssp.	1[f] terpene product
9	sAA9812	67, 69	<i>Xanthophyllomyces dendrorhous</i> XdCrtE <i>Cronobacter sakazakii</i> CsCrtB <i>Xanthophyllomyces dendrorhous</i> XdCrtI	HDE1	geranylgeranyl diphosphate synthase (CrtE), phytoene synthase (CrtB), phytoene desaturase (CrtI)	<i>Candida viswanthii</i>	lycopene
10	sAA9814	67, 69	<i>Xanthophyllomyces dendrorhous</i> XdCrtE <i>Cronobacter sakazakii</i> CsCrtB <i>Xanthophyllomyces dendrorhous</i> XdCrtI	HDE1	geranylgeranyl diphosphate synthase (CrtE), phytoene synthase (CrtB), phytoene desaturase (CrtI)	<i>Candida viswanthii</i>	lycopene

	Strain	Example No.	1[a] heterologous nucleic acids encoding	1[b]/1[d] promoter	1[c] terpene biosynthesis enzymes	1[e] <i>Candida</i> ssp or <i>Blastobotrys</i> ssp.	1[f] terpene product
11	sAA9817	68, 69	<i>Xanthophyllomyces dendrorhous</i> XdCrtE <i>Cronobacter sakazakii</i> CsCrtB <i>Xanthophyllomyces dendrorhous</i> XdCrtI	HDE1	geranylgeranyl diphosphate synthase (CrtE), phytoene synthase (CrtB), phytoene desaturase (CrtI)	<i>Candida viswanthii</i>	lycopene
12	sAA9819	68, 69	<i>Xanthophyllomyces dendrorhous</i> XdCrtE <i>Cronobacter sakazakii</i> CsCrtB <i>Xanthophyllomyces dendrorhous</i> XdCrtI	HDE1	geranylgeranyl diphosphate synthase (CrtE), phytoene synthase (CrtB), phytoene desaturase (CrtI)	<i>Candida viswanthii</i>	lycopene

	Strain	Example No.	1[a] heterologous nucleic acids encoding	1[b]/1[d] promoter	1[c] terpene biosynthesis enzymes	1[e] <i>Candida</i> ssp or <i>Blastobotrys</i> ssp.	1[f] terpene product
13	sAA9821	68, 69	<i>Xanthophyllomyces dendrorhous</i> XdCrtE <i>Cronobacter sakazakii</i> CsCrtB <i>Xanthophyllomyces dendrorhous</i> XdCrtI	HDE1	geranylgeranyl diphosphate synthase (CrtE), phytoene synthase (CrtB), phytoene desaturase (CrtI)	<i>Candida viswanthii</i>	lycopene
14	sAA9936	3/22/2023 affidavit	<i>Archaeoglobus fulgidus</i> AfCrtE <i>Cronobacter sakazakii</i> CsCrtB <i>Xanthophyllomyces dendrorhous</i> XdCrtI	POX	geranylgeranyl pyrophosphate synthase (CrtE), phytoene synthase (CrtB), phytoene desaturase (CrtI),	<i>Blastobotrys adeninivorans</i>	lycopene

	Strain	Example No.	1[a] heterologous nucleic acids encoding	1[b]/1[d] promoter	1[c] terpene biosynthesis enzymes	1[e] <i>Candida</i> ssp or <i>Blastobotrys</i> ssp.	1[f] terpene product
15	sVZ287	3/22/2023 affidavit			N/A – Affidavit and response does not provide information on this strain.	<i>Candida</i>	lycopene
16	sAA7453	36, 37	<i>Callitropsis nootkatensis</i> CnTPS1	HDE1	valencene synthase (TPS)	<i>Candida viswanthii</i>	valencene

206. Given that *Candida* is a genus containing over 430 species, a POSITA would recognize that the '148 Patent provides working examples in only 0.2% of the *Candida* species claimed. Despite the specifications providing working examples of fatty acid-induced terpene production in only one species of *Candida*, the '148 Patent claims the entire *Candida* genus.

207. Similarly, as discussed in ¶¶ 193-195 and 204, a declaration submitted by inventor Dr. Beardslee on March 22, 2023, provides a single working example in a *Blastobotrys* species. Otherwise, *Blastobotrys* is mentioned in the specification only among long, non-limited lists of microorganisms and embodiments; in a mention that *B. adenivorans* can use carbon- and nitrogen-containing compounds as a sole source of carbon and nitrogen; and in a few examples of untested embodiment permutations. As discussed above, the '148 Patent claims a broader class than it provides examples for, including by seeking to claim the entirety of the *Blastobotrys* genus on the basis of a single working example. Given that *Blastobotrys* is a genus containing over 20 species, the '148 Patent provides working examples in 5% of the *Blastobotrys* species claimed.

208. The '148 Patent also fails to provide adequate guidance to enable embodiments beyond those illustrated by working examples. Despite claiming tens of thousands of terpenes, the '148 Patent only provides working examples of fatty

acid- or alkane-induced production of three terpenes (and only carotenoid molecules more specifically): lycopene, β -carotene, and valencene. Ex. 1001, '148 Patent at p. 255 (Example 32), p. 255 (Example 33), p. 256 (Example 39), p. 257 (Example 40), p. 258 (Examples 45-49), p. 259 (Example 52), p. 259 (Example 54), p. 259 (Example 57), p. 260 (Example 58), p. 260 (Example 60), p. 260 (Example 61), pp. 261-262 (Examples 67-69 (for lycopene)), p. 258 (Examples 47-48 (for β -carotene)), and p. 256 (Examples 36-37 (for valencene)); and Ex. 1002, '148 PH at p. 3033 (Table 1 (for lycopene)).

209. By Dr. Beardslee's own admission, the changes to the organism or the biological pathway require reevaluation of the promoter to achieve the results claimed, in any fermentation system, promoters should be not only present but also, carefully selected based upon the *production organism* and the *fermentation conditions* present during production. If the promoters selected are effectively 'off' in the conditions present during fermentation, then no gene product (enzyme) will be made, *rendering the intended engineered biochemical pathway useless for production.*" (emphasis added) Ex. 1002, '148 PH at p. 2943 (¶ 11).

210. Furthermore, despite Dr. Beardslee's admission that the fermentation conditions present during production are a key aspect of selecting promoters and, by extension, in generating a construct capable of producing a terpene, the working examples of fatty acid- and alkane-induced terpene production illustrated in the '148

Patent largely focus on oleic acid as the inducer, with the exception of Example 49 (results in Table 22) showing lycopene production after induction by C10-C14 alkanes, nonane, and octadecane.

X. DOCUMENTS AND REFERENCES CONSIDERED

211. **Ex. 1007, Weaver 2012:** The book chapter, “The Molecular Nature of Genes,” and cover and bibliographic pages, by Weaver, are true and correct copies I retrieved of a chapter and excerpts (“Weaver 2012,” Ex. 1007) from the scientific textbook *Molecular Biology*, Fifth Edition, with ISBN: 978-0-07-352532-7 (“*Molecular Biology*”). The bibliographic page of the textbook from which the copy of this chapter was retrieved shows a copyright date of 2012 by The McGraw-Hill Companies. Ex. 1007 at 4. *Molecular Biology* is a textbook designed for, and commonly used and referenced in institutions of higher education, including for introductory courses in molecular biology. Institutions of higher education obtain textbooks like *Molecular Biology* on or shortly after their publication to sell and/or maintain in libraries for access and review. This chapter (Ex. 1007) from *Molecular Biology* therefore would have been accessible to a POSITA at an institute of higher education around the time of its publication in 2012 and well-before 2017. For example, *Molecular Biology* is used to teach undergraduate students well-known topics in genetics, DNA, and gene expression. A POSITA therefore would have been very familiar with *Molecular Biology*, would have had access to *Molecular Biology*

either through purchasing the textbook or through libraries or online, including at institutes of higher education, and would have reason to access Molecular Biology to research and understand topics and technologies in the technical area of the '148 Patent. I found and was able to access this textbook, Molecular Biology, including the chapter Weaver 2012, in The University of Texas at Austin Life Sciences Library Stacks. This textbook would have been readily accessible to a POSITA in The University of Texas at Austin Life Sciences Library Stacks or in other such libraries by 2017, and a POSITA would have had reason to consult such libraries for research and understanding in the technical area of the '148 Patent. I further reviewed Ex. 1056 (Hall-Ellis Exhibit 1007), and the content appears to be substantially the same (if not identical) to the content of Exhibit 1007 referenced throughout my declaration.

212. **Ex. 1008, Alberts 2008:** The book chapters, “Proteins,” “How Cells Read the Genome: From DNA to Protein,” and “Manipulating Proteins, DNA and RNA,” cover and bibliographic pages, and pages 415 and 739-742 by Alberts et al., are true and correct copies I retrieved of chapters and excerpts (“Alberts 2008,” Ex. 1008) from the scientific textbook Molecular Biology of THE CELL, Fifth Edition, with ISBN: 978-0-8153-4105-5 (“Molecular Biology of THE CELL”). The bibliographic page of the textbook from which the copies of these chapters and excerpts were retrieved shows a copyright date of 2008. Ex. 1008 at 6. Molecular Biology of THE CELL has been the leading cell biology textbook for nearly a

quarter century and is designed for, obtained by, and commonly used and referenced in institutes of higher education, including to provide a framework for understanding cell biology for non-expert readers. Institutions of higher education obtain textbooks like Molecular Biology of THE CELL on or shortly after their publication to sell and/or maintain in libraries for access and review. These chapters and excerpts (Ex. 1008) from Molecular Biology of THE CELL therefore would have been accessible to a POSITA at an institute of higher education around the time of its publication in 2008 and well before 2017. For example, Molecular Biology of THE CELL is a commonly used textbook in undergraduate and graduate courses relating to molecular biology. A POSITA therefore would have been very familiar with Molecular Biology of THE CELL, would have had access to Molecular Biology of THE CELL either through purchasing the textbook or through libraries or online, including at institutes of higher education, and would have reason to access Molecular Biology of THE CELL to research and understand topics and technologies in the technical area of the '148 Patent. I found and was able to access this textbook, Molecular Biology of THE CELL, including the chapters and excerpts in Alberts 2008, in The University of Texas at Austin Life Sciences Library Stacks. This textbook would have been readily accessible to a POSITA in The University of Texas at Austin Life Sciences Library Stacks or in other such libraries by 2017, and a POSITA would have had reason to consult such libraries for research and

understanding in the technical area of the '148 Patent. I further reviewed Ex. 1057 (Hall-Ellis Exhibit 1008), and the content appears to be substantially the same (if not identical) to the content of Exhibit 1008 referenced throughout my declaration.

213. **Ex. 1009, Maimone 2007:** The article, “Modern synthetic efforts toward biologically active terpenes,” by Maimone and Baran (“Maimone 2007,” Ex. 1009), is a true and correct copy I retrieved of an article from the scientific journal Nature Chemical Biology (part of the Nature publishing family), Volume 3, July 2007, with doi: 10.1038/nchembio2007.1 (“Nature Chemical Biology”). The face of the article as retrieved from Nature Chemical Biology and Google Scholar states that it was “Published online” on “18 June 2007,” and shows a copyright date of 2007. Ex. 1009 at 1. Nature Chemical Biology has been published since at least 2005 and is a well-known and peer reviewed publication with articles that are published monthly online at www.nature.com and indexed routinely. Maimone 2007 therefore would have been accessible to a POSITA around the time of Nature Chemical Biology’s and its publication in 2007 at least in its online form at www.nature.com and accessible via Google Scholar by 2017. Nature Chemical Biology is an international monthly journal publishing original research and commentary, like Maimone 2007, relevant to controlling biological processes at the molecular level and has a high impact score. A POSITA therefore would have been very familiar with Nature Chemical Biology, would have had access to Nature Chemical Biology

either through libraries or online, and would have had reason to access Nature Chemical Biology to research and understand topics and technologies in the technical area of the '148 Patent. I found and was able to access this article using the Google Scholar search tool, which is a tool myself and others in my field regularly use, and have used since at least 2017, for academic and scientific research. Google Scholar would have been readily accessible to a POSITA by 2017, and a POSITA would have had reason to use Google Scholar for research and understanding of the topics and technologies in the technical area of the '148 Patent. For example, I was able to access the full article through my university access point. I further reviewed Ex. 1058 (Hall-Ellis Exhibit 1009), and the content appears to be substantially the same (if not identical) to the content of Exhibit 1009 referenced throughout my declaration.

214. **Ex. 1010, Zwenger 2008:** The article, “Plant terpenoids: applications and future potentials,” by Zwenger and Basu (“Zwenger 2008,” Ex. 1010), is a true and correct copy I retrieved of an article from the scientific journal Biotechnology and Molecular Biology Reviews, Volume 3, February 2008, with doi: 10.5897/BMBR2008.0001 (“Biotechnology and Molecular Biology Reviews”). The face of the article as retrieved from Biotechnology and Molecular Biology Reviews and Google Scholar states that it was “Available online,” shows an issue date of February 2008, and shows a copyright date of 2008. Ex. 1010 at 1.

Biotechnology and Molecular Biology Reviews has been published since 2006 and is a peer-reviewed, open-access publication with articles focusing on areas such as biotechnology and molecular biology. Zwenger 2008 therefore would have been accessible to a POSITA around the time of its publication in 2008 at least in its online form at <https://academicjournals.org/journal/BMBR> and accessible via Google Scholar by 2017. A POSITA therefore would have had access to Biotechnology and Molecular Biology Reviews online, and would have had reason to access Biotechnology and Molecular Biology Reviews to research and understand topics and technologies in the technical area of the '148 Patent. I found and was able to access this article using the Google Scholar search tool, which is a tool myself and others in my field regularly use, and have used since at least 2017, for academic and scientific research and understanding in the technical area of the '148 Patent. Google Scholar would have been readily accessible to a POSITA by 2017, and a POSITA would have had reason to use Google Scholar for research. A link to the article is available at

<https://digscholarship.unco.edu/cgi/viewcontent.cgi?article=1003&context=biofacpub>. Wayback also shows that this article was indeed available at least by October

2017. *See, e.g.,*

<https://web.archive.org/web/20171019142011/https://digscholarship.unco.edu/cgi/viewcontent.cgi?article=1003&context=biofacpub>. For example, I was able to

access the full article through my university access point. I further reviewed Ex. 1059 (Hall-Ellis Exhibit 1010), and the content appears to be substantially the same (if not identical) to the content of Exhibit 1010 referenced throughout my declaration.

215. **Ex. 1011, Blazeck 2013:** The article, “Promoter engineering: Recent advances in controlling transcription at the most fundamental level,” by Blazeck and Alper (“Blazeck 2013,” Ex. 1011), is a true and correct copy I retrieved of an article from the scientific journal Biotechnology Journal, Volume 8, 2013, with doi: 10.1002/biot.201200120 (“Biotechnology Journal”). The face of the article as retrieved from Biotechnology Journal and Google Scholar shows a copyright date of 2012 and an issue date of 2013. Ex. 1011 at 1. Biotechnology Journal has been published since 2006 and is a well-known and peer reviewed publication with articles that is published monthly online at <https://analyticalsciencejournals.onlinelibrary.wiley.com/journal/18607314> and indexed routinely. Blazeck 2013 therefore would have been accessible to a POSITA around the time of Biotechnology Journal’s publication in 2013 at least in its online form at <https://analyticalsciencejournals.onlinelibrary.wiley.com/journal/18607314> and accessible via Google Scholar by 2017. Biotechnology Journal is a journal publishing research articles and reviews, like Blazeck 2013, in all areas of biotechnology and bioengineering research, and has a reasonably high impact score. A POSITA therefore would have been very familiar with Biotechnology Journal,

would have had access to Biotechnology Journal either through libraries or online, and would have had reason to access Biotechnology Journal to research and understand topics and technologies in the technical area of the '148 Patent. I co-authored this article and found and was able to access this article through the Google Scholar search tool, which is a tool myself and others in my field regularly use, and have used since at least 2017, for academic and scientific research. Google Scholar would have been readily accessible to a POSITA by 2017, and a POSITA would have had reason to use Google Scholar for research and understanding topics and technologies in the technical area of the '148 Patent. I was able to access the full article through my university access point. I further reviewed Ex. 1060 (Hall-Ellis Exhibit 1011), and the content appears to be substantially the same (if not identical) to the content of Exhibit 1011 referenced throughout my declaration.

216. **Ex. 1012, Weake 2010:** The article, “Inducible gene expression: diverse regulatory mechanisms,” by Weake and Workman (“Weake 2010,” Ex. 1012), is a true and correct copy I retrieved of an article from the scientific journal Nature Reviews Genetics (part of the Nature publishing family), Volume 11, June 2010, with doi: 10.1038/nrg2781 (“Nature Reviews Genetics”). The face of the article as retrieved from Nature Reviews Genetics and Google Scholar states that it was “Published online” on “27 April 2010,” and shows a copyright date of 2010. Ex. 1012 at 1. Nature Reviews Genetics has been published since 2000 and is a

well-known and peer-reviewed publication with articles that is published monthly online at www.nature.com and indexed routinely. Weake 2010 therefore would have been accessible to a POSITA around the time of Nature Reviews Genetics publication in 2010 at least in its online form at www.nature.com and accessible via Google Scholar by 2017. Nature Reviews Genetics is a journal publishing research highlights, comments, reviews, and perspectives, like Weake 2010, relevant to geneticists and genomicists and has a high impact factor. A POSITA therefore would have been very familiar with Nature Reviews Genetics, would have had access to Nature Reviews Genetics either through libraries or online, and would have had reason to access Nature Reviews Genetics to research and understand topics and technologies in the technical area of the '148 Patent. I found and was able to access this article using the Google Scholar search tool, which is a tool myself and others in my field regularly use, and have used since at least 2017, for academic and scientific research. Google Scholar would have been readily accessible to a POSITA by 2017, and a POSITA would have had reason to use Google Scholar for research and understanding in the technical area of the '148 Patent. A link to the article is available at <https://www.nature.com/articles/nrg2781>. For example, I was able to access the full article through my university access point. I further reviewed Ex. 1061 (Hall-Ellis Exhibit 1012), and the content appears to be substantially the same (if not

identical) to the content of Exhibit 1012 referenced throughout my declaration.

217. **Ex. 1013, Weinhandl 2010:** The article, “Carbon source dependent promoters in yeasts,” by Weinhandl et al. (“Weinhandl 2010,” Ex. 1013), is a true and correct copy I retrieved of an article from the scientific journal *Microbial Cell Factories*, Volume 13, 2014, with doi: 10.1186/1475-2859-13-5 (“*Microbial Cell Factories*”). The face of the article as retrieved from *Microbial Cell Factories* and Google Scholar states that it was “Published” on “9 January 2014,” and shows a copyright date of 2014. Ex. 1013 at 1, 13. *Microbial Cell Factories* has been published since at least 2002, is indexed routinely, and is accessible on BioMed Central, a well-known, open-access repository of journals supported by Springer Nature. Weinhandl 2010 therefore would have been accessible to a POSITA around the time of *Microbial Cell Factories* publication in 2014 at least in its online form at <https://microbialcellfactories.biomedcentral.com/> and accessible via Google Scholar by 2017. *Microbial Cell Factories* is a journal publishing peer-reviewed research, like Weinhandl 2010, focusing on applied microbiology and has a reasonably high impact factor. Moreover, I served as an Associate Editor for the journal *Microbial Cell Factories* from 2017-2022, so I can attest to the scientific rigor of peer review and reputation of the journal. A POSITA therefore would have been very familiar with *Microbial Cell Factories*, would have had access to *Microbial Cell Factories* either through libraries or online, and would have had reason to access *Microbial*

Cell Factories to research and understand topics and technologies in the technical area of the '148 Patent. I found and was able to access this article using the Google Scholar search tool, which is a tool myself and others in my field regularly use, and have used since at least 2017, for academic and scientific research. Google Scholar would have been readily accessible to a POSITA by 2017, and a POSITA would have had reason to use Google Scholar for research and understanding in the technical area of the '148 Patent. A link to the article is available at <https://link.springer.com/content/pdf/10.1186/1475-2859-13-5.pdf>. For example, I was able to access the full article through my university access point. I further reviewed Ex. 1062 (Hall-Ellis Exhibit 1013), and the content appears to be substantially the same (if not identical) to the content of Exhibit 1013 referenced throughout my declaration.

218. **Ex. 1014, Kanai 2000 / Ex. 1015, Kanai Fig. 3:** The article, “An *n*-Alkane-Responsive Promoter Element Found in the Gene Encoding the Peroxisomal Protein of *Candida tropicalis* Does Not Contain a C₆ Zinc Cluster DNA-Binding Motif,” by Kanai et al. (“Kanai 2000,” Ex. 1014), is a true and correct copy I retrieved of an article from the scientific journal *Journal of Bacteriology*, Volume 182, May 2000, with doi: 10.1128/jb.182.9.2492-2497.2000 (“*Journal of Bacteriology*”). Ex. 1015 (“Kanai Fig. 3”) is a true and correct enlarged copy of Figure 3 from Kanai 2000. Ex. 1014 at 5. The face of Kanai 2000 as retrieved from

Journal of Bacteriology and Google Scholar shows a copyright date of 2000 by the American Society for Microbiology and an issue date of May 2000. Ex. 1014 at 1. The Journal of Bacteriology has been published since 1916 and is a well-known and peer-reviewed publication with articles that is published monthly online at <https://journals.asm.org/journal/jb> and indexed routinely. Kanai 2000 and Kanai Fig. 3 therefore would have been accessible to a POSITA around the time of Journal of Bacteriology's publication in 2000 at least in its online form at <https://journals.asm.org/journal/jb> and accessible via Google Scholar by 2017. Journal of Bacteriology is a journal publishing primary-research articles, like Kanai 2000, related to bacteria, archaea, and their viruses. A POSITA therefore would have been familiar with the Journal of Bacteriology, would have had access to the Journal of Bacteriology either through libraries or online, and would have had reason to access the Journal of Bacteriology to research and understand topics and technologies in the technical area of the '148 Patent. I found and was able to access this article using the Google Scholar search tool, which is a tool myself and others in my field regularly use, and have used since at least 2017, for academic and scientific research. Google Scholar would have been readily accessible to a POSITA by 2017, and a POSITA would have had reason to use Google Scholar for research and understanding in the technical area of the '148 Patent. A link to the article is available at <https://journals.asm.org/doi/pdf/10.1128/jb.182.9.2492-2497.2000>. For

example, I was able to access the full article through my university access point. I further reviewed Ex. 1063 (Hall-Ellis Exhibit 1014), and the content appears to be substantially the same (if not identical) to the content of Exhibit 1014 referenced throughout my declaration. I further reviewed Ex. 1064 (Hall-Ellis Exhibit 1015), and Figure 3 of Ex. 1064 appears to be substantially the same (if not identical) to the content of Exhibit 1015 referenced throughout my declaration.

219. **Ex. 1016, Masuda 1995 / Ex. 1017, Masuda Fig. 2:** The article, “Cloning and characterization of the *POX2* gene in *Candida maltosa*,” by Masuda et al. (“Masuda 1995,” Ex. 1016), is a true and correct copy I retrieved of an article from the scientific journal *Gene*, Volume 167, December 1995, with doi: 10.1016/0378-1119(95)00655-9 (“Gene”). Ex. 1017 (“Masuda Fig. 2”) is a true and correct enlarged copy of Figure 2 from Masuda 1995. Ex. 1016 at 3. The face of Masuda 1995 shows a copyright date of 1995 by Elsevier Science B.V., and an issue date of 1995. Ex. 1016 at 1. *Gene* has been published since 1976 and is a well-known and peer-reviewed publication with articles that is published monthly online at <https://www.sciencedirect.com/journal/gene/> and indexed routinely. Masuda 1995 and Masuda Fig. 2 therefore would have been accessible to a POSITA around the time of *Gene*’s publication in 1995 and accessible via Google Scholar by 2017. *Gene* is a journal publishing papers, like Masuda 1995, focusing on the regulation, expression, function, and evolution of genes in all biological applications. A

POSITA therefore would have been familiar with Gene, would have had access to Gene either through libraries or online, and would have had reason to access Gene to research and understand topics and technologies in the technical area of the '148 Patent. I found and was able to access this article using the Google Scholar search tool, which is a tool myself and others in my field regularly use, and have used since at least 2017, for academic and scientific research and understanding in the area of the '148 Patent. Google Scholar would have been readily accessible to a POSITA by 2017, and a POSITA would have had reason to use Google Scholar for research. A link to the article is available at <https://www.sciencedirect.com/science/article/abs/pii/S0378111995006559?via%3Dihub>. For example, I was able to access the full article through my university access point. I further reviewed Ex. 1065 (Hall-Ellis Exhibit 1016), and the content appears to be substantially the same (if not identical) to the content of Exhibit 1016 referenced throughout my declaration. I further reviewed Ex. 1066 (Hall-Ellis Exhibit 1017), and Figure 2 of Ex. 1066 appears to be substantially the same (if not identical) to the content of Exhibit 1017 referenced throughout my declaration.

220. **Ex. 1018, Lee 2015:** The article, “A Highly Characterized Yeast Toolkit for Modular, Multipart Assembly,” by Lee et al. (“Lee 2015,” Ex. 1018), is a true and correct copy I retrieved of an article from the scientific journal ACS Synthetic Biology, Volume 4, 2015, with doi: 10.1021/sb500366v (“ACS Synthetic

Biology”). The face of the article as retrieved from ACS Synthetic Biology and Google Scholar states that it was “Published” on “April 14, 2015,” and shows a copyright date of 2015 by the American Chemical Society. Ex. 1018 at 1. ACS Synthetic Biology has been published since 2012 and is a well-known and peer-reviewed publication with articles that is published monthly online at <https://pubs.acs.org/journal/asbcd6>. Lee 2015 therefore would have been accessible to a POSITA around the time of ACS Synthetic Biology’s publication in 2015 at least in its online form and accessible via Google Scholar by 2017. ACS Synthetic Biology is a journal publishing research, like Lee 2015, across all areas of synthetic biology, including genomics and genome replacement strategies, and has a high impact factor. A POSITA therefore would have been very familiar with ACS Synthetic Biology, would have had access to ACS Synthetic Biology either through libraries or online, and would have had reason to access ACS Synthetic Biology to research and understand topics and technologies in the technical area of the ’148 Patent. I found and was able to access this article using the Google Scholar search tool, which is a tool myself and others in my field regularly use, and have used since at least 2017, for academic and scientific research. Google Scholar would have been readily accessible to a POSITA by 2017, and a POSITA would have had reason to use Google Scholar for research and understanding in the technical area of the ’148 Patent. A link to the article is available at

<https://pubs.acs.org/doi/pdf/10.1021/sb500366v>. For example, I was able to access the full article through my university access point. I further reviewed Ex. 1067 (Hall-Ellis Exhibit 1018), and the content appears to be substantially the same (if not identical) to the content of Exhibit 1018 referenced throughout my declaration.

221. **Ex. 1019, van der Walt 1980:** The article, “The yeast genus *Yarrowia* gen. nov.,” by van der Walt and von Arx (“van der Walt 1980,” Ex. 1019), is a true and correct copy I retrieved of an article from the scientific journal *Antonie van Leeuwenhoek*, Volume 46, November 1980, with doi: 10.1007/BF00394008 (“Antonie van Leeuwenhoek”). The article as retrieved from *Antonie van Leeuwenhoek* and Google Scholar is part of an issue dated 1980. Ex. 1019 at 1. *Antonie van Leeuwenhoek* has been published since 1934 and is a well-known and peer-reviewed publication with articles that is published monthly online at <https://link.springer.com/journal/10482> and indexed routinely. van der Walt 1980 therefore would have been accessible to a POSITA around the time of *Antonie van Leeuwenhoek*’s publication in 1980 and would have been accessible via Google Scholar by 2017. *Antonie van Leeuwenhoek* is a journal publishing papers, like van der Walt 1980, focusing on the fundamental and applied aspects of microbiology and including research in the areas of molecular biology and genetics. A POSITA therefore would have been very familiar with *Antonie van Leeuwenhoek*, would have had access to *Antonie van Leeuwenhoek* either through libraries or online, and

would have had reason to access Antonie van Leeuwenhoek to research and understand topics and technologies in the technical area of the '148 Patent. I found and was able to access this article using the Google Scholar search tool, which is a tool myself and others in my field regularly use, and have used since at least 2017, for academic and scientific research. Google Scholar would have been readily accessible to a POSITA by 2017, and a POSITA would have had reason to use Google Scholar for research and understanding in the technical area of the '148 Patent. For example, I was able to access the full article through my university access point. I further reviewed Ex. 1068 (Hall-Ellis Exhibit 1019), and the content appears to be substantially the same (if not identical) to the content of Exhibit 1019 referenced throughout my declaration.

222. **Ex. 1020, van der Walt 1990:** The article, “*Arxula* gen. nov. (Candidaceae), a new anamorphic, arthroconidial yeast genus,” by van der Walt et al. (“van der Walt 1990,” Ex. 1020), is a true and correct copy I retrieved of an article from the scientific journal Antonie van Leeuwenhoek, Volume 57, January 1990, with doi: 10.1007/BF00400338 (“Antonie van Leeuwenhoek”). The face of the article as retrieved from Antonie van Leeuwenhoek and Google Scholar shows a copyright date of 1990 by Kluwers Academic Publishers and is part of an issue dated 1990. Ex. 1020 at 1. Antonie van Leeuwenhoek is further discussed in ¶ 221. van der Walt 1990 therefore would have been accessible to a POSITA around the time

of Antonie van Leeuwenhoek's publication in 1990 and would have been accessible via Google Scholar by 2017. For example, I found this article using the Google Scholar search tool, which is a tool myself and others in my field regularly use, and have used since at least 2017, for academic and scientific research. Google Scholar would have been readily accessible to a POSITA by 2017, and a POSITA would have had reason to use Google Scholar for research and understanding in the technical area of the '148 Patent. For example, I was able to access the full article through my university access point. I further reviewed Ex. 1069 (Hall-Ellis Exhibit 1020), and the content appears to be substantially the same (if not identical) to the content of Exhibit 1020 referenced throughout my declaration.

223. **Ex. 1021, Craft 2003:** The article, "Identification and Characterization of the *CYP52* Family of *Candida tropicalis* ATCC 20336, Important for the Conversion of Fatty Acids and Alkanes to α,ω -Dicarboxylic Acids," by Craft et al. ("Craft 2003," Ex. 1021), is a true and correct copy I retrieved of an article from the scientific journal Applied and Environmental Microbiology, Volume 69, October 2003, with doi: 10.1128/AEM.69.10.5983–5991.2003 ("Applied and Environmental Microbiology"). The face of the article as retrieved from Applied and Environmental Microbiology and Google Scholar shows a copyright date of 2003 by the American Society for Microbiology and is from an issue dated October 2003. Ex. 1021 at 1. Applied and Environmental Microbiology has been published since 1953 and is a

well-known and peer-reviewed publication with articles that is published monthly online at <https://journals.asm.org/journal/aem> and indexed routinely. Craft 2003 therefore would have been accessible to a POSITA around the time of Applied and Environmental Microbiology's publication in 2003 and accessible via Google Scholar by 2017. Applied and Environmental Microbiology is a journal publishing papers, like Craft 2003, focusing on results of studies with significant contributions to applied microbial research, and genetic and molecular investigations of practically useful microbial topics. Applied and Environmental Microbiology has a reasonably high impact factor. Moreover, I have served as a member of the Editorial Board for the journal Applied and Environmental Microbiology since 2017, so I can attest to the scientific rigor of peer review and reputation of the journal. A POSITA therefore would have been very familiar with Applied and Environmental Microbiology, would have had access to Applied and Environmental Microbiology either through libraries or online, and would have had reason to access Applied and Environmental Microbiology to research and understand topics and technologies in the technical area of the '148 Patent. For example, I found this article using the Google Scholar search tool, which is a tool myself and others in my field regularly use, and have used since at least 2017, for academic and scientific research. Google Scholar would have been readily accessible to a POSITA by 2017, and a POSITA would have had reason to use Google Scholar for research and understanding in the technical area of

the '148 Patent. A link to the article is available at <https://journals.asm.org/doi/full/10.1128/aem.69.10.5983-5991.2003>. I was able to access the full article through my university access point. I further reviewed Ex. 1070 (Hall-Ellis Exhibit 1021), and the content appears to be substantially the same (if not identical) to the content of Exhibit 1021 referenced throughout my declaration.

224. **Ex. 1022, NCYC 825:** This vendor, National Collection of Yeast Cultures (NCYC) is a British yeast culture collection based at the Norwich Research Park in Norwich, Norfolk, United Kingdom and maintains a collection of over 4,400 yeast strains. The NCYC was founded in 1948 and is a well-known vendor that provides yeast cultures to researchers across the globe in both academic and industrial settings. A link to the NCYC is available at <https://www.ncyc.co.uk/>. The NCYC sells *Yarrowia lipolytica* strain NCYC 825, alternatively known as ATCC 8661 and NRRL Y-1095, which can be found at <https://www.ncyc.co.uk/catalogue/yarrowia-lipolytica-825>. NCYC 825 (*i.e.*, ATCC 8661) was deposited in December of 1975 by the American Type Culture Collection (ATCC), a nonprofit organization which collects, stores, and distributes standard reference microorganisms, cell lines and other materials for research and development. A POSITA would readily have had access to this strain and information concerning this strain by 2017, and a POSITA would have had reason to use this vendor to purchase or research the history and provenance of yeast strains

for research. I was able to access the full vendor website through my university access point. I further reviewed Ex. 1071 (Hall-Ellis Exhibit 1022), and the content appears to be substantially the same (if not identical) to the content of Exhibit 1022 referenced throughout my declaration.

225. **Ex. 1023, Kunze 2014:** The article, “The complete genome of *Blastobotrys (Arxula) adenivorans* LS3 - a yeast of biotechnological interest” and its supplemental datafile “Phylogeny and synteny of *A. adenivorans* and other yeasts” by Kunze et al. (“Kunze 2014,” Ex. 1023), are true and correct copies of an article and its supplemental datafile “Additional File 7” from the scientific journal *Biotechnology for Biofuels*, Volume 7, 2014, with doi: 10.1186/1754-6834-7-66 (“*Biotechnology for Biofuels*”). The face of the article as retrieved from *Biotechnology for Biofuels* and Google Scholar states that it was “Published” on “24 April 2014,” and shows a copyright date of 2014 by Kunze et al. and licensed to BioMed Central Ltd. Ex. 1023 at 1, 13. *Biotechnology for Biofuels*, renamed to *Biotechnology for Biofuels and Bioproducts* in 2022, has been published since at least 2009 and is accessible on BioMed Central, a well-known open-access repository of journals supported by Springer Nature. Kunze 2014 therefore would have been accessible to a POSITA around the time of *Biotechnology for Biofuels*’ publication in 2014 in its online form at <https://biotechnologyforbiofuels.biomedcentral.com/> and accessible via Google

Scholar by 2017. Biotechnology for Biofuels is a journal publishing peer-reviewed research, like Kunze 2014, focusing on advancements in understanding, technological capabilities, and production of biofuels, biochemicals, and biomaterials. Biotechnology for Biofuels has a high impact factor. A POSITA therefore would have been very familiar with Biotechnology for Biofuels, would have had access to Biotechnology for Biofuels either through libraries or online, and would have had reason to access Biotechnology for Biofuels to research and understand topics and technologies in the technical area of the '148 Patent. I found this article using the Google Scholar search tool, which is a tool myself and others in my field regularly use, and have used since at least 2017, for academic and scientific research. Google Scholar would have been readily accessible to a POSITA by 2017, and a POSITA would have had reason to use Google Scholar for research and understanding in the technical area of the '148 Patent. A link to the article is available at

<https://biotechnologyforbiofuels.biomedcentral.com/articles/10.1186/1754-6834-7-66>. Wayback also shows that this article was indeed available at least by December

15, 2016. See, e.g.,

<https://web.archive.org/web/20161225163900/https://biotechnologyforbiofuels.biomedcentral.com/articles/10.1186/1754-6834-7-66>. For example, I was able to access the full article through my university access point. I further reviewed Ex. 1072 (Hall-

Ellis Exhibit 1023), and the content appears to be substantially the same (if not identical) to the content of Exhibit 1023 referenced throughout my declaration.

226. **Ex. 1024, Middelhoven 1984:** The article, “*Trichosporon adeninovorans* sp. nov., a yeast species utilizing adenine, xanthine, uric acid, putrescine and primary *n*-alkylamines as the sole source of carbon, nitrogen and energy,” by Middelhoven et al. (“Middelhoven 1984,” Ex. 1024), is a true and correct copy I retrieved of an article from the scientific journal *Antonie van Leeuwenhoek*, Volume 50, July 1984, with doi: 10.1007/BF00394651 (“Antonie van Leeuwenhoek”). The face of the article as retrieved from *Antonie van Leeuwenhoek* and Google Scholar shows that it was part of an issue dated 1984. Ex. 1024 at 1. *Antonie van Leeuwenhoek* is further discussed in ¶ 221. Middelhoven 1984 therefore would have been accessible to a POSITA around the time of *Antonie van Leeuwenhoek*’s publication in 1984 and would have been accessible via Google Scholar by 2017. For example, I found this article using the Google Scholar search tool, which is a tool myself and others in my field regularly use, and have used since at least 2017, for academic and scientific research. Google Scholar would have been readily accessible to a POSITA by 2017, and a POSITA would have had reason to use Google Scholar for research and understanding in the technical area of the ’148 Patent. For example, I was able to access the full article through my university access point. I further reviewed Ex. 1073 (Hall-Ellis Exhibit 1024), and

the content appears to be substantially the same (if not identical) to the content of Exhibit 1024 referenced throughout my declaration.

227. **Ex. 1025, Kurtzman & Robnett 2007:** The article, “Multigene phylogenetic analysis of the *Trichomonascus*, *Wickerhamiella* and *Zygoascus* yeast clades, and the proposal of *Sugiyamaella* gen. nov. and 14 new species combinations,” by Kurtzman and Robnett (“Kurtzman & Robnett 2007,” Ex. 1025), is true and correct copy I retrieved of an article from the scientific journal FEMS Yeast Research, Volume 7, 2007, with doi: 10.1111/j.1567-1364.2006.00157.x (“FEMS Yeast Research”). The face of the article states that it was “First published online” on “12 September 2006,” and shows a copyright date of 2006 by the Federation of European Microbiological Societies. Ex. 1025 at 1. FEMS Yeast Research has been published since 2001 and is a well-known, open-access, and peer-reviewed publication with articles that are published yearly online at <https://academic.oup.com/femsyr>, indexed routinely, and accessible via Google Scholar by 2017. Moreover, I have served as a member of the Editorial Board for the journal FEMS Yeast Research since 2013, so I can attest to the scientific rigor of peer review and reputation of the journal. This article therefore would have been accessible to a POSITA around the time of its publication in 2006. FEMS Yeast Research publishes high-quality research articles, mini-reviews, letters, perspectives and commentaries, like Kurtzman & Robnett 2007, contributing to POSITA’s

understanding of biology and the impact of yeasts. A POSITA therefore would have been very familiar with FEMS Yeast Research, would have had access to FEMS Yeast Research either through libraries or online, and would have had reason to access FEMS Yeast Research to research and understand topics and technologies in the technical area of the '148 Patent. I found and was able to access this article using the Google Scholar search tool, which is a tool myself and others in my field regularly use, and have used since at least 2017, for academic and scientific research. Google Scholar would have been readily accessible to a POSITA by 2017, and a POSITA would have had reason to use Google Scholar for research and understanding of the topics and technologies in the technical area of the '148 Patent. A link to the article is available at <https://academic.oup.com/femsyr/article/7/1/141/492833>. For example, I was able to access the full article through my university access point. I further reviewed Ex. 1074 (Hall-Ellis Exhibit 1025), and the content appears to be substantially the same (if not identical) to the content of Exhibit 1025 referenced throughout my declaration.

228. **Ex. 1026, Kurtzman 2015:** The article, “Advances in yeast systematics and phylogeny and their use as predictors of biotechnologically important metabolic pathways,” by Kurtzman et al.(“Kurtzman 2015, ” Ex. 1026), is a true and correct copy I retrieved of an article from the scientific journal FEMS Yeast Research, Volume 15, 2015, with doi: 10.1093/femsyr/fov050 (“FEMS Yeast Research”). The

face of the article shows an “Advance Access Publication Date” of “1 July 2015,” shows that it was part of an issue from 2015, and states that it was “Published by Oxford University Press on behalf of FEMS 2015.” Ex. 1026 at 1. FEMS Yeast Research is further discussed in ¶ 227. This article therefore would have been accessible to a POSITA around the time of its publication in 2015. I found and was able to access this article using the Google Scholar search tool, which is a tool myself and others in my field regularly use, and have used since at least 2017, for academic and scientific research. Google Scholar would have been readily accessible to a POSITA by 2017, and a POSITA would have had reason to use Google Scholar for research and understanding of the topics and technologies in the technical area of the ’148 Patent. A link to the article is available at <https://academic.oup.com/femsyr/article/15/6/fov050/634871>. Moreover, I have served as a member of the Editorial Board for the journal FEMS Yeast Research since 2013, so I can attest to the scientific rigor of peer review and reputation of the journal. For example, I was able to access the full article through my university access point.

229. **Ex. 1027, de Hoog 2015:** The article, “Name Changes in Medically Important Fungi and Their Implications for Clinical Practice,” by de Hoog et al. (“de Hoog 2015,” Ex. 1027), is a true and correct copy I retrieved of an article from the scientific Journal of Clinical Microbiology, Volume 53, April 2015, with doi:

10.1128/JCM.02016-14 (“Journal of Clinical Microbiology”). The face of the article as retrieved from Journal of Clinical Microbiology and Google Scholar states that the “Accepted manuscript [was] posted online” on “8 October 2014,” and shows a copyright date of 2015 by the American Society for Microbiology. Ex. 1027 at 1. The Journal of Clinical Microbiology has been published since 1975 and is a well-known and peer-reviewed publication with articles that are published monthly online at <https://journals.asm.org/journal/jcm> and indexed routinely. de Hoog 2015 therefore would have been accessible to a POSITA around the time of Journal of Clinical Microbiology's and its publication in 2015 at least in its online form at <https://journals.asm.org/journal/jcm> and accessible via Google Scholar by 2017. The Journal of Clinical Microbiology is a journal publishing papers involving laboratory tests and results for the diagnosis and monitoring of infectious diseases, and typing methods and genome sequencing of pathogens. A POSITA therefore would have been very familiar with Journal of Clinical Microbiology, would have had access to Journal of Clinical Microbiology either through libraries or online, and would have reason to access Journal of Clinical Microbiology to research and understand topics and technologies in the technical area of the '148 Patent. I found this article using the Google Scholar search tool, which is a tool myself and others in my field regularly use, and have used since at least 2017, for academic and scientific research. Google Scholar would have been readily accessible to a POSITA by 2017, and a

POSITA would have had reason to use Google Scholar for research and understanding of the topics and technologies in the technical area of the '148 Patent. A link to the article is available at <https://journals.asm.org/doi/full/10.1128/jcm.02016-14>. For example, I was able to access the full article through my university access point.

230. **Ex. 1028, Daniel 2014:** The article, “On the reclassification of species assigned to *Candida* and other anamorphic ascomycetous yeast genera based on phylogenetic circumscription,” by Daniel et al. (“Daniel 2014,” Ex. 1028), is a true and correct copy I retrieved of an article from the scientific journal *Antonie van Leeuwenhoek*, Volume 106, April 2014, with doi: 10.1007/s10482-014-0170-z (“Antonie van Leeuwenhoek”). The face of the article as retrieved from *Antonie van Leeuwenhoek* and Google Scholar states that it was “Published online” on “19 April 2014,” and shows a copyright date of 2014 by Springer International Publishing Switzerland. Ex. 1028 at 1. *Antonie van Leeuwenhoek* is further discussed in ¶ 221. Daniel 2014 therefore would have been accessible to a POSITA around the time of *Antonie van Leeuwenhoek*’s publication in 2014 and accessible via Google Scholar by 2017. I found and was able to access this article using the Google Scholar search tool, which is a tool myself and others in my field regularly use, and have used since at least 2017, for academic and scientific research. Google Scholar would have been readily accessible to a POSITA by 2017, and a POSITA would have had reason to

use Google Scholar for research and understanding of the topics and technologies in the technical area of the '148 Patent. For example, I was able to access the full article through my university access point.

231. **Ex. 1029, Lachance 2011:** The book chapter, “*Candida* Berkhout (1923),” and cover and bibliography pages, by Lachance et al., are true and correct copies I retrieved of a chapter and excerpts (“Lachance 2011,” Ex. 1029) from the scientific book *The Yeasts, a Taxonomic Study*, Volume 2, Fifth Edition, with ISBN: 978-0-123-84707-2 (“*The Yeasts, a Taxonomic Study*”). The bibliographic page of the book from which the copy of this chapter was retrieved shows a copyright date of 2011 by Elsevier B.V. Ex. 1029 at 3. *The Yeasts, a Taxonomic Study* is a three-volume book providing information about the identification of yeasts and is commonly used by researchers, students, clinicians, taxonomists, and biotechnologists in the fields of microbiology, genetics, and molecular biology. Volume 2 of the book specifically focuses on the identification and classification of ascomycetous yeasts. Institutions of higher education obtain textbooks like *The Yeasts, a Taxonomic Study* on or shortly after their publication to sell and/or maintain in libraries for access and review. This chapter (Ex. 1029) from *The Yeasts, a Taxonomic Study* therefore would have been accessible to a POSITA at an institute of higher education around the time of its publication in 2011 and well before 2017. A POSITA would have been very familiar with *The Yeasts, a Taxonomic Study*,

would have had access to *The Yeasts, a Taxonomic Study* either through purchasing the textbook or through libraries or online, including at institutes of higher education, and would have reason to access *The Yeasts, a Taxonomic Study* to research topics and technologies in the technical area of the '148 Patent. I found and was able to access this book, *The Yeasts, a Taxonomic Study*, including the chapter Lachance 2011, through my university library, which I regularly use for academic and scientific research. This book would have been readily accessible to a POSITA in The University of Texas at Austin Life Sciences Library Stacks or in other such libraries by 2017, and a POSITA would have had reason to use libraries for research and understanding in the technical area of the '148 Patent. For example, I was able to access the full book through my university library

232. **Ex. 1030, Smith 2011:** The book chapter, “*Blastobotrys* von Klopotek (1967),” and cover and bibliographic pages, by Smith et al., are true and correct copies I retrieved of a chapter and excerpts (“Smith 2011,” Ex. 1030) from the scientific book *The Yeasts, a Taxonomic Study*, Volume 2, Fifth Edition, with ISBN: 978-0-123-84707-2 (“*The Yeasts, a Taxonomic Study*”). The bibliographic page of the book shows a copyright date of 2011. *The Yeasts, a Taxonomic Study* is further discussed in ¶ 231. This chapter (Ex. 1030) from *The Yeasts, a Taxonomic Study* therefore would have been accessible to a POSITA at an institute of higher education around the time of its publication in 2011 and well before 2017. I found and was

able to access this book, *The Yeasts, a Taxonomic Study*, including the chapter Smith 2011, through my university library, which I regularly use for academic and scientific research. This book would have been readily accessible to a POSITA in The University of Texas at Austin Life Sciences Library Stacks or in other such libraries by 2017, and a POSITA would have had reason to use such libraries for research and understanding in the technical area of the '148 Patent. For example, I was able to access the full book through my university library.

233. **Ex. 1031, Bailey 1991:** The article, “Toward a Science of Metabolic Engineering,” by Bailey (“Bailey 1991,” Ex. 1031), is a true and correct copy I retrieved of an article from the scientific journal *Science*, Volume 252, 1991, with doi: 10.1126/science.2047876 (“Science”). *Science* has been published since 1880 and is a well-known and peer-reviewed journal published by the American Association for the Advancement of Science. *Science* is published with articles weekly online at <https://www.science.org/journal/science> and is indexed routinely. Bailey 1991 therefore would have been accessible to a POSITA around the time of *Science* and its publication in 1991 at least in its online form at www.science.org and accessible via Google Scholar by 2017. *Science* publishes high-quality research, like Bailey 1991, across all areas of science and its articles are consistently ranked among the most cited globally, the journal having a high impact factor. A POSITA therefore would have been very familiar with *Science*, would have had access to

Science either through libraries or online, and would have had reason to access Science to research and understand topics and technologies in the technical area of the '148 Patent. I found and was able to access this article using the Google Scholar search tool, which is a tool myself and others in my field regularly use for academic and scientific research, and have used since at least 2017. Google Scholar would have been readily accessible to a POSITA by 2017, and a POSITA would have had reason to use Google Scholar for research and understanding of the topics and technologies in the technical area of the '148 Patent. For example, I was able to access the full article through my university access point.

234. **Ex. 1032 and Ex. 1033, Barns 1991:** The article, “Evolutionary Relationships among Pathogenic *Candida* Species and Relatives,” by Barns et al. (“Barns 1991,” Ex. 1032), is a true and correct copy I retrieved of an article from the scientific Journal of Bacteriology, Volume 173, April 1991, with doi: 10.1128/jb.173.7.2250-2255.1991 (“Journal of Bacteriology”). Ex. 1033 is a true and correct copy of Table 3 from Barns 1991. The face of Barns 1991 shows a copyright date of 1991 by the American Society for Microbiology, and that the article was part of an issue from April 1991. Ex. 1032 at 1. The Journal of Bacteriology is further discussed in ¶ 218. Barns 1991 therefore would have been accessible to a POSITA around the time of Journal of Bacteriology’s publication in 1991 and accessible via Google Scholar by 2017. I found and was able to access this

article using the Google Scholar search tool, which is a tool myself and others in my field regularly use, and have used since at least 2017, for academic and scientific research. Google Scholar would have been readily accessible to a POSITA by 2017, and a POSITA would have had reason to use Google Scholar for research and understanding of the topics and technologies in the technical area of the '148 Patent. A link to the article is available at <https://journals.asm.org/doi/abs/10.1128/jb.173.7.2250-2255.1991>. For example, I was able to access the full article through my university access point.

235. **Ex. 1034, Beopoulos 2008:** The article, “Control of Lipid Accumulation in the Yeast *Yarrowia lipolytica*,” by Beopoulos et al. (“Beopoulos 2008,” Ex. 1034), is a true and correct copy I retrieved of an article from the scientific journal Applied and Environmental Microbiology, Volume 74, December 2008, with doi: 10.1128/AEM.01412-08 (“Applied and Environmental Microbiology”). The face of the article as retrieved from Applied and Environmental Microbiology and Google Scholar states that it was “Published ahead of print on 24 October 2008,” and shows a copyright date of 2008 by the American Society for Microbiology. Ex. 1034 at 1. Applied and Environmental Microbiology is further discussed in ¶ 223. Beopoulos 2008 therefore would have been accessible to a POSITA around the time of Applied and Environmental Microbiology’s publication in 2008 and accessible via Google Scholar by 2017. I found and was able to access

this article using the Google Scholar search tool, which is a tool myself and others in my field regularly use, and have used since at least 2017, for academic and scientific research. Google Scholar would have been readily accessible to a POSITA by 2017, and a POSITA would have had reason to use Google Scholar for research and understanding of the topics and technologies in the technical area of the '148 Patent. A link to the article is available at <https://journals.asm.org/doi/full/10.1128/aem.01412-08>. For example, I was able to access the full article through my university access point.

236. **Ex. 1035 and Ex. 1036, Dujon 2010:** The article, “Yeast evolutionary genomics,” by Dujon (“Dujon 2010,” Ex. 1035), is a true and correct copy I retrieved of an article from the scientific journal Nature Reviews Genetics, Volume 11, July 2010, with doi: 10.1038/nrg2811 (“Nature Reviews Genetics”). Ex. 1036 is a true and correct copy of Figure 1 from Dujon 2010. The face of Dujon 2010 as retrieved from Nature Reviews Genetics and Google Scholar shows a copyright date of 2010 by Macmillan Publishers Limited, and shows that it was part of an issue from July 2010. Ex. 1035 at 1. Nature Reviews Genetics is further discussed in ¶ 216. Dujon 2010 therefore would have been accessible to a POSITA around the time of Nature Reviews Genetics’ publication in 2010 and accessible via Google Scholar by 2017. I found and was able to access this article using the Google Scholar search tool, which is a tool myself and others in my field regularly use, and have used since at

least 2017, for academic and scientific research. Google Scholar would have been readily accessible to a POSITA by 2017, and a POSITA would have had reason to use Google Scholar for research and understanding of the topics and technologies in the technical area of the '148 Patent. For example, I was able to access the full article through my university access point.

237. **Ex. 1037, Gabaldon 2016:** The article, “Evolutionary genomics of yeast pathogens in the Saccharomycotina,” by Gabaldon et al. (“Gabaldon 2016,” Ex. 1037), is a true and correct copy I retrieved of an article from the scientific journal FEMS Yeast Research, Volume 16, 2016, with doi: 10.1093/femsyr/fow064 (“FEMS Yeast Research”). The face of the article as retrieved from FEMS Yeast Research and Google Scholar shows a copyright date of 2016 by FEMS, shows an advance access publication date of 3 August 2016, and shows that it was part of an issue from 2016. Ex. 1037 at 1. FEMS Yeast Research is further discussed in ¶ 227. Gabaldon 2016 therefore would have been accessible to a POSITA around the time of FEMS Yeast Research’s publication in 2016 and accessible via Google Scholar by 2017. I found and was able to access this article using the Google Scholar search tool, which is a tool myself and others in my field regularly use, and have used since at least 2017, for academic and scientific research. Google Scholar would have been readily accessible to a POSITA by 2017, and a POSITA would have had reason to use Google Scholar for research and understanding of the topics and technologies in

the technical area of the '148 Patent. As described above, I have served as a member of the Editorial Board for the journal FEMS Yeast Research since 2013, so I can attest to the scientific rigor of peer review and reputation of the journal. A link to the article is available at <https://academic.oup.com/femsyr/article/16/6/fow064/2198008>. For example, I was able to access the full article through my university access point.

238. **Ex. 1038, Khunnamwong 2015:** The article, “Description of *Diutina* gen. nov., *Diutina siamensis*, f.a. sp. nov., and reassignment of *Candida catenulata*, *Candida mesorugosa*, *Candida neorugosa*, *Candida pseudorugosa*, *Candida ranongensis*, *Candida rugosa* and *Candida scorzettiae* to the genus *Diutina*,” by Khunnamwong et al. (“Khunnamwong 2015,” Ex. 1038), is a true and correct copy I retrieved of an article from the scientific International Journal of Systematic and Evolutionary Microbiology (IJSEM), Volume 65, 2015, with doi: 10.1099/ijsem.0.000634 (“IJSEM”). The face of the article as retrieved from IJSEM and Google Scholar shows a copyright date of 2015 to International Union of Microbiological Societies (IUMS), and that it was part of an issue from 2015. Ex. 1038 at 1. IJSEM has been published since at least 1951 and is a well-known peer reviewed publication with articles that are published monthly online at <https://www.microbiologyresearch.org/> and indexed routinely. Khunnamwong 2015 therefore would have been accessible to a POSITA around the time of IJSEM’s

and its publication in 2015 at least in its online form at <https://www.microbiologyresearch.org/> and accessible via Google Scholar by 2017. IJSEM is an international monthly journal publishing original research and commentary, like Khunnamwong 2015, relevant to microbial evolution, phylogenetics and systematics. A POSITA therefore would have been very familiar with IJSEM, would have had access to IJSEM either through libraries or online, and would have had reason to access IJSEM to research and understand topics and technologies in the technical area of the '148 Patent. I found and was able to access this article using the Google Scholar search tool, which is a tool myself and others in my field regularly use, and have used since at least 2017, for academic and scientific research. Google Scholar would have been readily accessible to a POSITA by 2017, and a POSITA would have had reason to use Google Scholar for research and understanding of the topics and technologies in the technical area of the '148 Patent. A link to the article is available at <https://www.microbiologyresearch.org/content/journal/ijsem/10.1099/ijsem.0.000634>. For example, I was able to access the full article through my university access point.

239. **Ex. 1039, Kidd 2023:** The article, “Fungal Nomenclature: Managing Change is the Name of the Game,” by Kidd et al. (“Kidd 2023,” Ex. 1039), is a true and correct copy I retrieved of an article from the scientific journal Open Forum

Infectious Diseases (OFID), Volume 10, January 2023, with doi: 10.1093/ofid/ofac559 (“OFID”). The face of the article as retrieved from OFID and Google Scholar shows a copyright date of 2023 by the authors, and published by Oxford University Press on behalf of OFID. Ex. 1039 at 1. OFID has been published since at least 2014 and is a well-known and peer reviewed publication with articles that are published monthly online at <https://academic.oup.com/ofid> and indexed routinely. OFID is a monthly international journal publishing original research and commentary, like Kidd 2023, relevant to the field of infectious diseases. Kidd 2023 is used to illustrate the historical changes in the field rather than serving as an example of contemporary literature. Kidd 2023 is accessible to a POSITA in its online form at <https://academic.oup.com/ofid>. A POSITA therefore would be familiar with OFID, would have access to OFID either through libraries or online, and would have had reason to access OFID to research and understand topics and technologies in the technical area of the '148 Patent. I found and was able to access this article using the Google Scholar search tool, which is a tool myself and others in my field regularly use, and have used since at least 2017, for academic and scientific research. Google Scholar would have been readily accessible to a POSITA by 2017, and a POSITA would have reason to use Google Scholar for research and understanding of the topics and technologies in the technical area of the '148 Patent.

A link to the article is available at

<https://academic.oup.com/ofid/article/10/1/ofac559/6974385>. For example, I was able to access the full article through my university access point.

240. **Ex. 1040, Kreger-van Rij 1984:** The book chapters, “Discussion of the Genera Belonging to the Ascosporegenous Yeasts,” (Chapter 3) and "Discussion of the Genera Belonging to the Imperfect Yeasts," (Chapter 5) and cover and bibliographic pages, edited by Kreger-van Rij, are true and correct copies I retrieved of chapters and excerpts (“Kreger-van Rij 1984,” Ex. 1040) from the scientific book *The Yeasts: a Taxonomic Study*, Third Edition, 2011, with ISBN: 0-444-80421-8 (“*The Yeasts: a Taxonomic Study*”). The copyright page of the book states that it was published in 1984 and has a copyright date of 1984 by Elsevier Science Publishers B.V. Ex. 1040 at 6. Elsevier Science Publishers B.V. have been publishing books since 1880 and can be found online at <https://www.elsevier.com/>. Institutions of higher education obtain textbooks like *The Yeasts: a Taxonomic Study* on or shortly after their publication to sell and/or maintain in libraries for access and review. These book chapters (Ex. 1040) therefore would have been accessible to a POSITA at an institute of higher education or in its online form at <https://www.elsevier.com/>. *The Yeasts: a Taxonomic Study* describes new developments in yeast taxonomy. A POSITA therefore would have been very familiar with *The Yeasts: a Taxonomic Study*, would have had access to *The Yeasts: a Taxonomic Study* either through purchasing the textbook or through libraries or

online, including at institutes of higher education, and would have reason to access The Yeasts: a Taxonomic Study to research topics in the technical area of the '148 Patent. I found and was able to access this book, The Yeasts: a Taxonomic Study, including the chapters Kreger-van Rij 1984, in my personal library, which I regularly use for academic and scientific research. This book would have been readily accessible to a POSITA in The University of Texas at Austin Life Sciences Library Stacks or in such other libraries by 2017, and a POSITA would have had reason to use libraries for research and understanding in the technical area of the '148 Patent.

241. **Ex. 1041, Kurtzman 2003:** The article, “Phylogenetic circumscription of *Saccharomyces*, *Kluyveromyces* and other members of the Saccharomycetaceae, and the proposal of the new genera *Lachancea*, *Nakaseomyces*, *Naumovia*, *Vanderwaltozyma* and *Zygorhizula*,” by Kurtzman (“Kurtzman 2003,” Ex. 1041), is a true and correct copy I retrieved of an article from the scientific journal FEMS Yeast Research, Volume 4, December 2003, with doi: 10.1016/S1567-1356(03)00175-2 (“FEMS Yeast Research”). The face of the article as retrieved from FEMS Yeast Research and Google Scholar states that it was “First published online” and shows a copyright date of 2003 by Federation of European Microbiological Societies and published by Elsevier B.V. Ex. 1041 at 1. FEMS Yeast Research is further discussed in ¶ 227. Kurtzman 2003 therefore would have been accessible to a POSITA around the time of FEMS Yeast Research’s publication

in 2003 and accessible via Google Scholar by 2017. I found and was able to access this article using the Google Scholar search tool, which is a tool myself and others in my field regularly use, and have used since at least 2017, for academic and scientific research. Google Scholar would have been readily accessible to a POSITA by 2017, and a POSITA would have had reason to use Google Scholar for research and understanding of the topics and technologies in the technical area of the '148 Patent. A link to the article is available at <https://academic.oup.com/femsyr/article/4/3/233/562841>. For example, I was able to access the full article through my university access point.

242. **Ex. 1042, Millerioux 2011:** The article, “Drug-resistant cassettes for the efficient transformation of *Candida guilliermondii* wild-type strains,” by Millerioux et al. (“Millerioux 2011,” Ex. 1042), is a true and correct copy I retrieved of an article from the scientific journal FEMS Yeast Research, Volume 11, September 2011, with doi: 10.1111/j.1567-1364.2011.00731.x (“FEMS Yeast Research”). The face of the article as retrieved from FEMS Yeast Research and Google Scholar states that the “Final version [was] published online” on “9 May 2011,” and shows a copyright date of 2011 by the Federation of European Microbiological Societies and published by Blackwell Publishing Ltd. Ex. 1042 at 1. FEMS Yeast Research is further discussed in ¶ 227. Millerioux 2011 therefore would have been accessible to a POSITA around the time of FEMS Yeast Research’s

publication in 2011 and accessible via Google Scholar by 2017. I found and was able to access this article using the Google Scholar search tool, which is a tool myself and others in my field regularly use, and have used since at least 2017, for academic and scientific research. Google Scholar would have been readily accessible to a POSITA by 2017, and a POSITA would have had reason to use Google Scholar for research and understanding of the topics and technologies in the technical area of the '148 Patent. A link to the article is available at <https://academic.oup.com/femsyr/article/11/6/457/492132>. For example, I was able to access the full article through my university access point.

243. **Ex. 1043, Papon 2012:** The article, “*Deus ex Candida* genetics: overcoming the hurdles for the development of a molecular toolbox in the CTG clade,” by Papon et al. (“Papon 2012,” Ex. 1043), is an article from the scientific journal *Microbiology*, Volume 158, March 2012, with doi: 10.1099/mic.0.055244-0 (“Microbiology”). The face of the article as retrieved from *Microbiology* and Google Scholar shows a copyright date of 2012 and that it was part of an issue dated 2012. Ex. 1043 at 1. *Microbiology* has been published since 1947 and is a peer-reviewed, open-access publication with articles that are published monthly online at <https://www.microbiologyresearch.org/content/journal/micro>. Papon 2012 therefore would have been accessible to a POSITA around the time of *Microbiology*’s and its publication in 2012 at least in its online form at

<https://www.microbiologyresearch.org/content/journal/micro> and accessible via Google Scholar by 2017. Microbiology is an international monthly journal publishing original research, like Papon 2012, focusing on areas of microbiology that address current global challenges, such as food security, environmental sustainability, and health. A POSITA therefore would have been very familiar with Microbiology, would have had access to Microbiology either through libraries or online, and would have had reason to access Microbiology to research and understand topics and technologies in the technical area of the '148 Patent. I found and was able to access this article using the Google Scholar search tool, which is a tool myself and others regularly use, and have used since at least 2017, for academic and scientific research. Google Scholar would have been readily accessible to a POSITA by 2017, and a POSITA would have had reason to use Google Scholar for research and understanding of the topics and technologies in the technical area of the '148 Patent. A link to the article is available at <https://www.microbiologyresearch.org/content/journal/micro/10.1099/mic.0.055244-0>. For example, I was able to access the full article through my university access point.

244. **Ex. 1044, Papon 2013:** The article, “Emerging and Emerged Pathogenic *Candida* Species: Beyond the *Candida albicans* Paradigm,” by Papon et al. (“Papon 2013,” Ex. 1044), is a true and correct copy I retrieved of an article from

the scientific journal PLOS Pathogens, Volume 9, September 2013, with doi: 10.1371/journal.ppat.1003550 (“PLOS Pathogens”). The face of the article as retrieved from PLOS Pathogens and Google Scholar shows a copyright date of 2013 and that the article was “Published September 26, 2013.” Ex. 1044 at 1. PLOS Pathogens has been published since 2005 and is a well-known open access journal with articles that are published monthly online at <https://journals.plos.org/plospathogens/>. Papon 2013 therefore would have been accessible to a POSITA around the time of PLOS Pathogen’s and its publication in 2013 at least in its online form at <https://journals.plos.org/plospathogens/> and accessible via Google Scholar by 2017. PLOS Pathogens includes scientific research, like Papon 2013, focused on pathogen biology, including viruses, bacteria, parasites, fungi, and prions and has a reasonably high impact factor. A POSITA therefore would have been very familiar with PLOS Pathogens, would have had access to PLOS Pathogens either through libraries or online, and would have had reason to access PLOS Pathogens to research and understand topics and technologies in the technical area of the ’148 Patent. I found and was able to access this article using the Google Scholar search tool, which is a tool myself and others in my field regularly use, and have used since at least 2017, for academic and scientific research. Google Scholar would have been readily accessible to a POSITA by 2017, and a POSITA would have had reason to use Google Scholar for research and understanding of the

topics and technologies in the technical area of the '148 Patent. A link to the article is available at <https://journals.plos.org/plospathogens/article?id=10.1371/journal.ppat.1003550&title=journals.plos.org>. For example, I was able to access the full article through my university access point.

245. **Ex. 1045, Paramasivan 2017:** The article, “Progress in terpene synthesis strategies through engineering of *Saccharomyces cerevisiae*,” by Paramasivan and Mutturi (“Paramasivan 2017,” Ex. 1045), is a true and correct copy I retrieved of an article from the scientific journal Critical Reviews in Biotechnology, Volume 37, 2017, with doi: 10.1080/07388551.2017.1299679 (“Critical Reviews in Biotechnology”). The face of the article as retrieved from Critical Reviews in Biotechnology and Google Scholar shows a copyright date of 2017 by Informa UK Limited, trading as Taylor & Francis Group, and shows that it was part of an issue dated 2017. Ex. 1045 at 1. Critical Reviews in Biotechnology has been published since at least 1983 and is a well-known peer reviewed publication with articles that are published quarterly online at <https://www.tandfonline.com/journals/ibty20>. Paramasivan 2017 therefore would have been accessible to a POSITA around the time of Critical Review in Biotechnology’s and its publication in 2017 in its online form at <https://www.tandfonline.com/journals/ibty20> and accessible via Google Scholar by 2017. Critical Reviews in Biotechnology is an international journal

publishing original research on biological techniques, like Paramasivan 2017, from fermentation to genetic manipulation, have become increasingly relevant to the food and beverage, fuel production, chemical and pharmaceutical, and waste management industries and has a reasonably high impact factor. A POSITA therefore would have been very familiar with Critical Reviews in Biotechnology, would have had access to Critical Reviews in Biotechnology either through libraries or online, and would have had reason to access Critical Reviews in Biotechnology to research and understand topics and technologies in the technical area of the '148 Patent. I found and was able to access this article using the Google Scholar search tool, which is a tool myself and others in my field regularly use, and have used since at least 2017, for academic and scientific research. Google Scholar would have been readily accessible to a POSITA by 2017, and a POSITA would have had reason to use Google Scholar for research and understanding of the topics and technologies in the technical area of the '148 Patent. A link to the article is available at https://www.researchgate.net/profile/Kalaivani-Paramasivan-2/publication/316313038_Progress_in_terpene_synthesis_strategies_through_engineering_of_Saccharomyces_cerevisiae/links/5afad616458515c00b6c371f/Progress-in-terpene-synthesis-strategies-through-engineering-of-Saccharomyces-cerevisiae.pdf. For example, I was able to access the full article through my university access point.

246. **Ex. 1046, Suh 2006:** The article, “Phylogenetics of Saccharomycetales, the ascomycete yeasts,” by Suh et al. (“Suh 2006,” Ex. 1046), is an article from the scientific journal *Mycologia*, Volume 98, 2006, with doi: 10.1080/15572536.2006.11832629 (“*Mycologia*”). The face of the article as retrieved from *Mycologia* and Google Scholar shows a copyright date of 2006 by The Mycological Society of America and shows that it was part of an issue from 2006. Ex. 1046 at 1. The article also shows that it was “accepted for publication 13 August 2006.” *Id.* *Mycologia* has been published since at least 1909 and is a well-known and peer reviewed publication with articles that are published six times a year online at <https://www.tandfonline.com/journals/umyc20>. Suh 2006 therefore would have been accessible to a POSITA around the time of *Mycologia*’s and its publication in 2006 at least in its online form at <https://www.tandfonline.com/journals/umyc20> and accessible via Google Scholar by 2017. *Mycologia* publishes research, like Suh 2006, in mycology, emphasizing all aspects of the biology of Fungi and fungus-like organisms, including lichens, oomycetes and slime molds. A POSITA therefore would have been very familiar with *Mycologia*, would have had access to *Mycologia* either through libraries or online, and would have had reason to access *Mycologia* to research and understand topics and technologies in the technical area of the ’148 Patent. I found and was able to access this article using the Google Scholar search tool, which is a tool myself

and others in my field regularly use, and have used since at least 2017, for academic and scientific research. Google Scholar would have been readily accessible to a POSITA by 2017, and a POSITA would have had reason to use Google Scholar for research and understanding of the topics and technologies in the technical area of the '148 Patent. For example, I was able to access the full article through my university access point.

247. **Ex. 1047, Takashima 2022:** The article, “Taxonomy of Pathogenic Yeasts *Candida*, *Cryptococcus*, *Malassezia*, and *Trichosporon*: Current Status, Future Perspectives, and Proposal for Transfer of Six *Candida* Species to the Genus *Nakaseomyces*,” by Takashima and Sugita (“Takashima 2022,” Ex. 1047), is an article from the scientific journal Medical Mycology Journal, Volume 63, 2022, with doi: 10.3314/mmj.22.004 (“Medical Mycology Journal”). The article has a copyright date of 2022 by the Japanese Society for Medical Mycology and shows that it was part of an issue from 2022. Medical Mycology Journal has been published since at least 2011 and is a peer reviewed publication with articles that are published quarterly online at <https://www.jstage.jst.go.jp/browse/mmj/-char/ja>. Takashima 2022 therefore is accessible to a POSITA in its online form at <https://www.jstage.jst.go.jp/browse/mmj/-char/ja>. Medical Mycology Journal is an international quarterly journal publishing original research on topics related to medical and veterinary mycology. This article is used to illustrate the historical

changes in the field rather than serving as an example of contemporary literature. A POSITA would be familiar with Medical Mycology Journal, would have access to Medical Mycology Journal either through libraries or online, and would have had reason to access Medical Mycology Journal to research and understand topics and technologies in the technical area of the '148 Patent. I found and was able to access this article using the Google Scholar search tool, which is a tool myself and others in my field regularly use, and have used since at least 2017, for academic and scientific research. Google Scholar is readily accessible to POSITAs and a POSITA would have reason to use Google Scholar for research and understanding of the topics and technologies in the technical area of the '148 Patent. A link to the article is available at https://www.jstage.jst.go.jp/article/mmj/63/4/63_22.004/_article/-char/ja/. For example, I was able to access the full article through my university access point.

248. **Ex. 1048, Yamada 2012:** The book chapter, “Diversity and Analysis of Bacterial Terpene Synthases,” and cover and bibliographic pages, by Yamada et al., are true and correct copies I retrieved of chapter 7 and excerpts (“Yamada 2012,” Ex. 1048) from the scientific book Methods in Enzymology, Volume 515, with doi: 10.1016/B978-0-12-394290-6.00007-0 (“Methods in Enzymology”). The bibliographic page of the book from which the copy of this chapter was retrieved shows a copyright date of 2012 by Elsevier Inc. and is published by ScienceDirect.

Ex. 1048 at 1. ScienceDirect has been publishing scientific and medical publications since 1997, including more than 4,000 academic journals and 30,000 e-books that are available online at <https://www.sciencedirect.com/>. Institutions of higher education obtain textbooks like Methods in Enzymology on or shortly after their publication to sell and/or maintain in libraries for access and review. This book chapter (Ex. 1048) from Methods in Enzymology therefore would have been accessible to a POSITA at an institute of higher education around the time of its publication in 2012 and also well before 2017 in its online form at <https://www.sciencedirect.com/>. A POSITA would have been very familiar with ScienceDirect, and had access to Methods in Enzymology either through purchasing the textbook or through libraries or online, including at institutes of higher education, and would have reason to access Methods in Enzymology to research and understand topics and technologies in the technical area of the '148 Patent. I found and was able to access this chapter from Methods in Enzymology, including Yamada 2012, in my personal library, which I regularly use for academic and scientific research. This chapter would have been readily accessible to a POSITA in The University of Texas at Austin Life Sciences Library Stacks or in other such libraries by 2017, and a POSITA would have had reason to use such libraries for research and understanding in the technical area of the '148 Patent.

249. **Ex. 1049, Zhang 2010:** The book chapter, “Heterologous Protein

Expression in Yeasts and Filamentous Fungi,” and cover and bibliographic pages, by Zhang and An, are a true and correct copies I retrieved of chapter 11 and excerpts (“Zhang 2010,” Ex. 1049) from the scientific book Manual of Industrial Microbiology and Biotechnology, Third Edition, 2010, with ISBN: 978-1-55581-512-7 (“Manual of Industrial Microbiology and Biotechnology”). The book shows a copyright date of 2010 by ASM Press. Ex. 1049 at 4. ASM Press has published books since at least 1899 and is co-published by Wiley Online Library at <https://onlinelibrary.wiley.com/>. Institutions of higher education obtain textbooks like Manual of Industrial Microbiology and Biotechnology on or shortly after their publication to sell and/or maintain in libraries for access and review. This chapter (Ex. 1049) from The Manual of Industrial Microbiology and Biotechnology therefore would have been accessible to a POSITA at an institute of higher education around the time of its publication in 2010, and also well-before 2017, in its online form at <https://onlinelibrary.wiley.com/>. For example, the Manual of Industrial Microbiology and Biotechnology includes an array of methods and discussions of microbial processes. A POSITA therefore would have been familiar with the Manual of Industrial Microbiology and Biotechnology, would have had access to the Manual of Industrial Microbiology and Biotechnology either through purchasing the textbook or through libraries or online, including at institutes of higher education, and would have reason to access the Manual of Industrial Microbiology and

Biotechnology to research and understand topics and technologies in the technical area of the '148 Patent. I found and was able to access this chapter, the Manual of Industrial Microbiology and Biotechnology, including the chapter Zhang 2010 in my personal library, which I regularly use for academic and scientific research. This chapter would have been readily accessible to a POSITA in The University of Texas at Austin Life Sciences Library Stacks or in other such libraries by 2017, and a POSITA would have had reason to use Google Scholar for research and understanding in the technical area of the '148 Patent.

250. **Ex. 1075, Sloots 1991 / Ex. 1076, Sloots Fig. 1:** The article, “Glucose-responsive and oleic acid-responsive elements in the gene encoding the peroxisomal trifunctional enzyme of *Candida tropicalis*,” by Sloots et al.(“Sloots 1991, ” Ex. 1075), is a true and correct copy I retrieved of an article from the scientific journal Gene, Volume 105, 1991, with doi: 10.1016/0378-1119(91)90524-F (“Gene”). Ex. 1076 (“Sloots Fig. 1”) is a true and correct enlarged copy of Figure 1 from Sloots 1991. Ex. 1075 at 2. The face of Sloots 1991 shows a copyright date of 1991 by Elsevier Science Publishers B.V., and an issue date of 1991, Ex. 1075 at 1. Gene is further discussed in ¶ 219. Sloots 1991 therefore would have been accessible to a POSITA around the time of its publication in 1991. I found and was able to access this article using the Google Scholar search tool, which is a tool myself and others in my field regularly use, and have used since at least 2017, for academic and

scientific research. Google Scholar would have been readily accessible to a POSITA by 2017, and a POSITA would have had reason to use Google Scholar for research and understanding of the topics and technologies in the technical area of the '148 Patent. A link to the article is available at <https://www.sciencedirect.com/science/article/abs/pii/037811199190524F>. For example, I was able to access the full article through my university access point.

XI. DECLARATION AND OATH

251. In signing this declaration, I understand that the declaration will be filed as evidence in a contested case before the Patent Trial and Appeal Board of the United States Patent and Trademark Office.

252. I acknowledge that I may be subject to cross-examination in this case and that cross-examination will take place within the United States. If cross-examination is required of me, I will appear for cross-examination within the United States during the time allotted for cross-examination. If a deposition is required of me, I will appear for the deposition at a suitable location to attend the same.

253. I declare that all statements made herein of my knowledge are true, and that all statements made on information and belief are believed to be true, and that these statements were made with the knowledge that willful false statements and the

like so made are punishable by fine or imprisonment, or both, under § 1001 of Title 18 of the United States Code.

Date: July 9, 2024



Hal S. Alper, Ph.D.

ANNEX A

Hal Alper

Kenneth A. Kobe Professor of Chemical Engineering
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The University of Texas at Austin
Department of Chemical Engineering
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I.	Education	VII.	Research
II.	Professional Experience	A.	Publications
III.	Teaching Experience	B.	Book Chapters
IV.	Memberships/Affiliations	C.	Patents and Patent Applications
V.	Selected Honors	D.	Presentations/Invited Talks
VI.	Editorial Duties	E.	Students Supervised
		VIII.	Service

I. Education

Postdoctorate, Mammalian Cell Engineering, 2007-2008

Shire Human Genetic Therapies, Inc., Cambridge, MA

Topic: Engineering of mammalian systems for protein production

Advisor: Yas Saotome

Postdoctorate, Yeast Genetics, 2006-2008

Whitehead Institute for Biomedical Research, Cambridge, MA

Topic: Engineering of yeast systems for the production of biofuels

Advisor: Gerry Fink

Ph.D., Chemical Engineering, 2006

Massachusetts Institute of Technology, Cambridge, MA

Topic: Development of Systematic and Combinatorial Approaches for the Metabolic Engineering of Microorganisms

Advisor: Gregory Stephanopoulos

B.S., Chemical Engineering, 2002

University of Maryland, College Park, College Park, MD

Summa Cum Laude

II. Professional Experience

2021-present Executive Director, Center for Biomedical Research Support
2018-2020 Associate Chair (Dept. Chem Eng, U. Texas at Austin)
2017-present Professor (Dept. Chem Eng, U. Texas at Austin)
2014-2017 Associate Professor (Dept. Chem Eng, U. Texas at Austin)
2013-2018 Graduate Advisor (Dept. Chem Eng, U. Texas at Austin)
2008-2014 Assistant Professor (Dept. Chem Eng, U. Texas at Austin)
2008-present Member, Institute for Cell and Molecular Biology (U. Texas at Austin)
2007-2008 Postdoctoral Research Associate (Shire Human Genetic Therapies)
2006-2008 Postdoctoral Research Associate (Whitehead Institute)
2002-2006 Graduate Research Assistant (MIT)
1999-2002 Undergraduate Research Assistant (University of Maryland)

III. Teaching Experience

Introduction to Chemical Engineering Analysis, ChE317 (UT)
Semesters Taught: Fall 2008, Spring 2009, Fall 2013, Fall 2016, Spring 2017,
Spring 2019, Spring 2020
Course Evaluation Summary: Average OIR of 4.57 and OCR of 4.13

Engineering Biology: From Biofuels to Biomedicine, UGS302 (UT)
Semesters Taught: Spring 2011, Spring 2012, Spring 2013, Spring 2014, Spring 2015,
Spring 2016, Fall 2018
Course Evaluation Summary: Average OIR of 4.76 and OCR of 4.6

Chemical Reactor Analysis and Design, ChE372 (UT)
Semesters Taught: Spring 2010, Fall 2010, Fall 2011, Fall 2015
Course Evaluation Summary: Average OIR of 4.4 and OCR of 3.85

Introduction to Computing, ChE210 (UT) (new required course)
Semesters Taught: Fall 2017, Spring 2018
Course Evaluation Summary: Average OIR of 3.45 and OCR of 2.85

Biochemical, Cellular, and Metabolic Engineering: Principles and Practices, ChE379/384 (UT)
Semesters Taught: Fall 2012
Course Evaluation Summary: Average OIR of 4.8 and OCR of 4.6

Prior to UT

2003-2004 Teaching Assistant, Kinetics of Biological and Chemical Systems (MIT)
2000 Undergrad. Teaching Assistant, Computer Methods in ChemE (UMCP)

IV. Memberships/Affiliations

2018-present Member, American Association for the Advancement of Science
2015-present Senior Member, American Institute of Chemical Engineers

2014-present Graduate Studies Committee, Microbiology Graduate Program, U Texas at Austin
 2012-present Member, American Society for Engineering Education, Deans Program Recipient
 2012-present Member, Society for Industrial Microbiology and Biotechnology
 2010-present Graduate Studies Committee, Department of Chemistry and Biochemistry,
 Biochemistry Division, U Texas at Austin
 2009-present Affiliate, UT Environmental Science Institute
 2008-present Member, Center for Synthetic and Systems Biology, U Texas at Austin
 2008-present Graduate Studies Committee, Cellular and Molecular Biology, U Texas at Austin
 2003-2015 Member, American Institute of Chemical Engineers
 2003-present Member, American Chemical Society

V. Selected Honors

2024 Fellow, American Association for the Advancement of Science
 2024 Fellow, International Academy of Medical and Biological Engineers
 2023 AIChE Andreas Acrivos Award for Professional Progress in Chemical Engineering
 2023 John van Geuns Lecturer, University of Amsterdam
 2023 UT Signature Course Distinguished Faculty
 2020 AIChE 2020 Fundraiser of the Year
 2019 Wiley Biosciences Award
 2019 AIChE Division 15 Service Award
 2019 AIChE Area 15c Plenary Speaker
 2019 Fellow, National Academy of Inventors
 2019 Edith and Peter O'Donnell Award in Engineering
 2019 Biochemical Engineering Journal Young Investigator Award
 2018 AIChE Allan P. Colburn Award for Excellence in Publications by a Young Member of
 the Institute
 2018 College of Fellows, The American Institute for Medical and Biological Engineering
 2018 Zhang Dayu Young Investigator Lectureship
 2018 *Molecular Systems Design & Engineering* Journal Emerging Investigator
 2017 Stratis V. Sotirchos Memorial Lectureship
 2017 Visiting Professor, Jiangnan University, China, School of Biotechnology
 2016 The University of Texas at Austin Emerging Inventor of the Year Award
 2016 American Chemical Society BIOT Division Young Investigator Award
 2015 Mellichamp Lecture, Purdue University, Department of Chemical Engineering
 2015 Society for Industrial Microbiology and Biotechnology Young Investigator Award
 2014 Jay Bailey Young Investigator Award in Metabolic Engineering
 2014 Camille Dreyfus Teacher-Scholar Award
 2014 Finalist, Dream Chemistry Award
 2013 Fellow of the Paul D. & Betty Robertson Meek Centennial Professorship (2013-current)
 2013 Invited Participant, NAE U.S. Frontiers of Engineering Symposium
 2013 *Biotechnology and Bioengineering* Daniel I.C. Wang Award
 2012 Regents' Outstanding Teaching Award (selected by the UT System)
 2011 Chevron Centennial Teaching Fellowship in Chemical Engineering (2011-2013)
 2011 Office of Naval Research Young Investigator Award

- 2011 Invited Participant, Karles Invitational Conference, Microbial Systems and Synthetic Biology
- 2010 DuPont Young Professor Award
- 2009 Invited Participant, National Academies Keck Futures Initiative (NAKFI) Conference, Synthetic Biology: Building on Nature's Inspiration
- 2009 Protégé, The 6th Annual Conference of The Academy of Medicine, Engineering & Science of Texas (TAMEST)--attendance based on nomination by National Academy (NAE) Member
- 2008 Texas Exes Teaching Award
- 2008 Camille and Henry Dreyfus New Faculty Award
- 2004 Merck Student/Young Investigator Poster Award for Best Poster (Metabolic Engineering Conference)

VI. Editorial Duties

- 2022-present Associate Editor for journal *Biotechnology and Bioengineering*
- 2021-present Associate Editor for journal *Biotechnology Advances*
- 2020-present Editorial Board for journal *Synthetic Biology*
- 2020-present Editorial Board for journal *Discover Chemical Engineering*
- 2018-present Founding Editor-in-Chief for journal *Biotechnology Notes*
- 2018-present Associate Editor for journal *Synthetic and Systems Biotechnology*
- 2017-2022 Senior Editor for the journal *Biotechnology Journal*
- 2017-present Editorial Board Member for the journal *Applied Microbiology and Biotechnology*
- 2017-2022 Associate Editor for the journal *Microbial Cell Factories*
- 2017 Guest Editor, Special Issue on "Metabolic science: Driving bioindustry and beyond" in *Biotechnology Journal*, published in 2017
- 2015-2018 Editorial Board Member for journal *Synthetic and Systems Biotechnology*
- 2013-present Reviews Editor for journal *Metabolic Engineering*
- 2013-2016 Editorial Board Member for the journal *FEMS Yeast Research*
- 2015 Guest Editor, Special Issue on "Yeast Synthetic Biology" in *FEMS Yeast Research*, published in 2015
- 2013 Guest Editor, Special Issue on "Systems Metabolic Engineering" in *Biotechnology Journal*, published in 2013
- 2012-2013 Associate Editor for the journal *Metabolic Engineering*
- 2012-2019 Associate Editor for the journal *BMC Systems Biology*
- 2012-2021 Associate Editor for the *Computational and Structural Biotechnology Journal*
- 2011 Book Series Editor, "Systems Metabolic Engineering: Methods and Protocols", Methods in Molecular Biology, Volume 985, 2013. ISBN: 9781627032988
- 2010 Guest Editor, Tissue, Cell and Pathway Engineering Issue in *Current Opinions in Biotechnology*, October 2012 Issue
- 2009 Lead Guest Editor, Special Issue "Applications of Synthetic Biology in Microbial Biotechnology", *Journal of Biomedicine and Biotechnology*, April 2010 Issue, ISSN: 1110-7243

VII. Research

A. Publications (As of 7/2/24, Google Scholar: 17,612 citations, h-index: 68)

1. Amelia Bergeson, Ashli Silvera, and Hal Alper, *Bottlenecks in biobased approaches to plastic degradation*, **Nature Communications**, Accepted/In Press.
2. Kevin Reed, Sierra Brooks, Jordan Wells, Kristin Blake, Minye Zhao, Kira Placido, Simon d'Oelsnitz, Adit Trivedi, Shruti Gadhiyar, and Hal Alper, *A Modular and Synthetic Biosynthesis Platform for de novo Production of Diverse Halogenated Tryptophan-Derived Molecules*, **Nature Communications**, 15, 3188, April 2024.
3. Kristin Presnell, Omar Melhem, Sarah Coleman, Nicholas Morse, and Hal Alper, 2024, *Design and synthesis of synthetic promoters for consistency of gene expression across growth phases and scale in S. cerevisiae*, **Synthetic and Systems Biotechnology**, Accepted/In Press.
4. Kevin Reed, Simon d'Oelsnitz, Sierra Brooks, Jordan Wells, Minye Zhao, Adit Trivedi, Selina Eshraghi, and Hal Alper, 2024, *Fluorescence-based Screens for engineering enzymes linked to halogenated tryptophan*, **ACS Synthetic Biology**, Accepted/In Press.
5. Celeste Marsan, Sung Gyung Lee, Ankim Nguyen, Angela Gordillo-Sierra, Sarah Coleman, Sierra Brooks, and Hal Alper, 2024, *Leveraging a Yarrowia lipolytica naringenin chassis for biosynthesis of apigenin and associated glucoside isovitexin*, **Metabolic Engineering**, Accepted/In Press.
6. Andree E. Ramirez-Partida, Luis Fernando Amador-Castro, Tomás García-Cayuela, Hal Alper, and Danay Carrillo-Nieves, *Towards a biorefinery processing Sargassum seaweed: Techno-economic assessment of alginate and fucoidan production through SuperPro Designer® process simulation*, **Environmental Technology & Innovation**, Accepted/In Press.
7. Simon d'Oelsnitz, Daniel Diaz, Mason Schechter, Daniel Acosta, Matthew Minus, James Howard, James Loy, Hannah Do, Hal Alper, and Andrew Ellington, 2024, *Biosensor and Machine Learning-Aided Engineering of an Amaryllidaceae Enzyme*, **Nature Communications**, Accepted/In Press.
8. Gokce Altin-Yavuzarslan, Naroa Sadaba, Sierra Brooks, Hal Alper, and Alshakim Nelson, 2023, *Engineered Living Material Bioreactors with Tunable Mechanical Properties using Vat Photopolymerization*, **Small**, Accepted/In Press.
9. Daniel Acosta and Hal Alper, 2023, *Advances in enzymatic and organismal technologies for the recycling and upcycling of petroleum-derived plastic waste*, **Current Opinion in Biotechnology**, 84:103021, December, 2023.
10. Xiunan Yi, Blake Rasor, Nathalie Boadi, Katherine Louie, Trent Northen, Ashty Karim, Michael Jewett, and Hal Alper, 2023, *Establishing a versatile toolkit of flux enhanced strains and cell extracts for pathway prototyping*, **Metabolic Engineering**, 80, 241-253, November 2023.
11. Sierra Brooks, Celeste Marsan, Kevin Reed, Shuo-Fu Yuan, Dustin-Dat Nguyen, Adit Trivedi, Gokce Altin-Yavuzarslan, Nathan Ballinger, Alshakim Nelson, and Hal Alper, *A tripartite microbial co-culture system for de novo biosynthesis of diverse plant phenylpropanoids*, **Nature Communications**, 14, 4448 (2023), July 2023.

12. Widiанти Sugianto, Gokce Altin-Yavuzarslan, Benjamin Tickman, Cholpsit Kiattisewee, Shuo-Fu Yuan, Sierra Brooks, Jitkanya Wong, Hal Alper, Alshakim Nelson, and James Carothers, *Gene expression dynamics in input-responsive engineered living materials programmed for bioproduction*, **Materials Today Bio**, 20, 100677, June 2023.
13. Emily Bowman, Phuong Nguyen Hoang, Angela Gordillo Sierra, Karoline Nogueira, and Hal Alper, 2023, *Temporal sorting of microdroplets can identify productivity differences of itaconic acid from libraries of Yarrowia lipolytica*, **Lab on a Chip**, 23, 2249 – 2256, May 2023.
14. Ya-Hue Valerie Soong, Sarah Coleman, Na Liu, Jiansong Qin, Carl Lawton, Hal Alper, and Dongming Xie, 2023, *Using Oils and Fats to Replace Sugars as Feedstocks for Biomanufacturing: Challenges and Opportunities for the Yeast Yarrowia lipolytica*, **Biotechnology Advances**, 65, 108128, July 2023.
15. Blake Rasor, Ashty Karim, Hal Alper, and Michael Jewett, 2023, *Cell extracts from bacteria and yeast retain metabolic activity after extended storage and repeated thawing*, **ACS Synthetic Biology**, 12(3), 904–908, February 2023.
16. Gokce Altin-Yavuzarslan, Sierra Brooks, Shuo-Fu Yuan, James Park, Hal Alper, and Alshakim Nelson, 2023, *Additive Manufacturing of Engineered Living Materials with Bio-Augmented Mechanical Properties and Resistance to Degradation*, **Advanced Functional Materials**, 330(24), 2300332, March 2023.
17. Sarah Coleman, Lauren Cordova, Beena Lad, Sabah Ali, Esha Ramanan, James Collett, and Hal Alper, 2023, *Evolving tolerance of Yarrowia lipolytica to hydrothermal liquefaction aqueous phase waste*, **Applied Microbiology and Biotechnology**, 107, 2011–2025, January 2023.
18. Sierra Brooks, Kevin Reed, Shuo-Fu Yuan, Ryan Shafranek, Gokce Altin-Yavuzarslan, Alshakim Nelson, and Hal Alper, 2023, *Enhancing long-term storage and stability of engineered living materials through desiccant storage and trehalose treatment*, **Biotechnology and Bioengineering**, 120(2), 572-582.
19. Angela R. Gordillo Sierra, Luis Fernando Amador-Castro, Andréé E. Ramírez-Partida, Tomás García-Cayuela, Danay Carrillo-Nieves, and Hal Alper, 2022, *Valorization of Caribbean Sargassum biomass as a source of alginate and sugars for de novo biodiesel production*, **Journal of Environmental Management**, 324, 116364, December 2022.
20. Shuo-Fu Yuan, Priya Nair, Dominic Borbon, Sarah Coleman, Po-Hsun Fan, Wen-Ling Lin, and Hal Alper, 2022, *Metabolic engineering of E. coli for β -alanine production using a multi-biosensor enabled approach*, **Metabolic Engineering**, 74, 24-35, November 2022.
21. Kristin Presnell, Omar Melhem, Nicholas Morse, and Hal Alper, 2022, *Modular, synthetic Boolean logic gates enabled in Saccharomyces cerevisiae through T7 polymerases / CRISPR dCas9 designs*, **ACS Synthetic Biology**, 11(10), 3414–3425, October 2022.
22. Cheng Cheng, Wei-Bin Wang, Meng-Lin Sun, Rui-Qi Tang, Long Bai, Hal Alper and Xinqing Zhao, 2022, *Deletion of NGG1 in the recombinant Saccharomyces cerevisiae improved xylose utilization and affected transcription of genes related to amino acid metabolism*, **Frontiers in Microbiology**, 13:960114, September 2022.
23. Simon d'Oelsnitz, Wantae Kim, Nathaniel Burkholder, Kamyab Javanmardi, Ross Thyer, Yan Zhang, Hal Alper, Andrew Ellington, 2022, *Using fungible biosensors to evolve improved alkaloid biosyntheses*, **Nature Chemical Biology**, 18, 981–989, July 2022.

24. Hongyuan Lu, Daniel Diaz, Natalie Czarnecki, Congzhi Zhu, Wantae Kim, Raghav Shroff, Daniel Acosta, Brad Alexander, Hannah Cole, Yan Jessie Zhang, Nathaniel Lynd, Andrew Ellington, and Hal Alper, 2022, *Machine learning-aided engineering of hydrolases for PET depolymerization*, **Nature**, 604, 662–667, April 2022.
25. Xiunan Yi and Hal Alper, 2022, *Considering strain variation and non-type strains for yeast metabolic engineering applications*, **Life**, 12(4), 510, April 2022.
26. Shenghu Zhou, Hal Alper, Jingwen Zhou, and Yu Deng, 2022, *Intracellular biosensor-based dynamic regulation to manipulate gene expression at the spatiotemporal level*, **Critical Reviews in Biotechnology**, 10.1080/07388551.2022.2040415, 1-18, April 2022.
27. Beena Lad, Sarah Coleman, and Hal Alper, 2022, *Microbial valorization of underutilized and nonconventional waste streams*, **Journal of Industrial Microbiology and Biotechnology**, 49(2), kuab056, March 2022.
28. Kelly Miller, Sarah Springthorpe, Jennifer Imbrogno, David Walker, Shruti Gadiyar, Benjamin Keitz, Andrew Ellington, Nathaniel Lynd, and Hal Alper 2022, *Biocompatible materials enabled by bio-based production of pyomelanin isoforms using an engineered Yarrowia lipolytica*, **Advanced Functional Materials**, 32(7), 2109366, February 2022.
29. Lauren Cordova, Claire Palmer, and Hal Alper, 2022, *Shifting the Distribution: Modulation of the Lipid Profile in Yarrowia lipolytica via Iron Content*, **Applied Microbiology and Biotechnology**, 106(4), 1571-1581, February 2022.
30. Jorge Gabriel Orozco-González, Fernando Amador-Castro, Angela Gordillo-Sierra, Tomás García-Cayuela, Hal Alper, and Danay Carrillo-Nieves, 2022, *Opportunities surrounding the use of Sargassum biomass as precursor of biogas, bioethanol, and biodiesel production*, **Frontiers in Marine Science**, 8:791054, January 2022.
31. Simon d'Oelsnitz, Vylan Nguyen, Hal Alper, Andrew Ellington, 2022, *Evolving a generalist biosensor for bicyclic monoterpenes*, **ACS Synthetic Biology**, 11(1), 265–272, January 2022.
32. Linfeng Xu, Kai-Chun Chang, Emory Payne, Cyrus Modavi, Leqian Liu, Claire Palmer, Nannan Tao, Hal Alper, Robert Kennedy, Dale Cornett and Adam Abate, 2021, *Mapping enzyme catalysis with metabolic biosensing*, **Nature Communications**, 12, 6803 (2021), November 2021.
33. Emily Bowman, James Wagner, Shuo-Fu Yuan, Matt Deaner, Claire Palmer, Simon D'Oelsnitz, Lauren Cordova, Xin Li, Frank F. Craig and Hal Alper, *Sorting for secreted molecule production using a biosensor-in-microdroplet approach*, **PNAS**, 118 (36) e2106818118, September 2021.
34. Shenghu Zhou, Shuo-Fu Yuan, Priya Nair, Hal Alper, Yu Deng, and Jingwen Zhou, *Development of a growth coupled and multi-layered dynamic regulation network balancing malonyl-CoA node to enhance (2S)-naringenin biosynthesis in Escherichia coli*, **Metabolic Engineering**, 67, 41-52, September 2021.
35. Blake Rasor, Xiunan Yi, Hunter Brown, Hal Alper, and Michael Jewett, *An integrated in vivo/in vitro framework to enhance cell-free biosynthesis with metabolically rewired yeast extracts*, **Nature Communications**, 12, 5139 (2021), August 2021.
36. Lauren Cordova, Hannah Walton, Eletria Biswas, Khristal Monroe, Rafael Quirino, and Hal Alper, *A data-driven approach to decipher the role of triglyceride composition on thermo-mechanical properties of thermosetting polymers using vegetable and microbial oils*, **ACS Applied Polymer Materials**, 3(9), 4485–4494, August 2021.

37. Shuo-Fu Yuan, Sierra Brooks, Annalee Nguyen, Wen-Ling Lin, Trevor Johnston, Jennifer Maynard, Alshakim Nelson, and Hal Alper, *Bioproduced Proteins On Demand (Bio-POD) in hydrogels using Pichia pastoris*, **Bioactive Materials**, 6(8), 2390-2399, August 2021.
38. Fernando Amador-Castro, Tomás García-Cayuela, Hal Alper, Veronica Rodriguez-Martinez, and Danay Carrillo-Nieves, *Valorization of pelagic Sargassum biomass into sustainable applications: current trends and challenges*, **Journal of Environmental Management**, 283, April 2021.
39. Sierra Brooks and Hal Alper, 2021, *Applications, challenges, and needs for employing synthetic biology beyond the lab*, **Nature Communications**, 12, 1390 (2021), March 2021.
40. Lichao Sun, Fengjiao Xin, and Hal Alper, 2021, *Bio-synthesis of food additives and colorants-a growing trend in future food*, **Biotechnology Advances**, 47, 107694, March 2021.
41. Angela Gordillo Sierra and Hal Alper, *Progress in the metabolic engineering of bio-based lactams and their ω -amino acids precursors*, **Biotechnology Advances**, 43, 107587, November 2020.
42. Lauren Cordova, Beena Lad, Sabah Ali, Andrew Schmidt, Justin Billing, Kyle Pomraning, Beth Hofstad, Marie Swita, James Collett, and Hal Alper, *Valorizing a Hydrothermal Liquefaction Aqueous Phase through co-production of chemicals and lipids using the oleaginous yeast Yarrowia lipolytica*, **Bioresource Technology**, 313, 123639, October 2020.
43. Rui-Qi Tang, James M. Wagner, Hal S. Alper, Xin-Qing Zhao, and Feng-Wu Bai, 2020, *Design, evolution and characterization of a xylose biosensor in Escherichia coli using the XylR/xylO system with an expanded operating range*, **ACS Synthetic Biology**, 9 (10), 2714–2722, September 2020.
44. Emily Bowman, Matthew Deaner, Jan-Fang Cheng, Robert Evans, Ernst Oberortner, Yasuo Yoshikuni, and Hal Alper, 2020, *Bi-directional Titration of Yeast Gene Expression using a Pooled CRISPR Guide RNA Approach*, **PNAS**, 117 (31) 18424-18430, August 2020.
45. Lichao Sun and Hal Alper, *Non-conventional hosts for the production of fuels and chemicals*, **Current Opinion in Chemical Biology**, 59, 15-22, December 2020.
46. Emily Bowman and Hal Alper, *Microdroplet-Assisted Screening of Biomolecule Production for Metabolic Engineering Applications*, **Trends in Biotechnology**, 38(7), 701-714, July 2020.
47. Shuo-Fu Yuan, Xiunan Yi, Trevor Johnston, and Hal Alper, *De novo resveratrol production through modular engineering of an Escherichia coli–Saccharomyces cerevisiae co-culture*, **Microbial Cell Factories**, 19, 143 (2020), July 2020.
48. Lauren Cordova, Jonathan Butler, and Hal Alper, *Direct production of fatty alcohols from glucose using engineered strains of Yarrowia lipolytica*, **Metabolic Engineering Communications**, 10, e00105, June 2020.
49. Trevor Johnston, Shuo-Fu Yuan, James Wagner, Xiunan Yi, Abhijit Saha, Patrick Smith, Alshakim Nelson and Hal Alper, *Compartmentalized microbes and co-cultures in hydrogels for on-demand bioproduction and preservation*, **Nature Communications**, 11, 563 (2020), February 2020.

50. Haibo Li and Hal Alper, *Producing biochemicals in Yarrowia lipolytica from xylose through a strain mating approach*, **Biotechnology Journal**, 15(2), 1900304, February 2020.
51. Claire Palmer, Kelly Miller, Ankim Nguyen, and Hal Alper, *Engineering 4-coumaroyl-CoA derived polyketide production in Yarrowia lipolytica through a β -oxidation mediated strategy*, **Metabolic Engineering**, 57, 174-181, January 2020.
52. Shuo-Fu Yuan and Hal Alper, *Improving Spinach2- and Broccoli-based biosensors for single and double analytes*, **Biotechnology Notes**, 1, 2-8, January 2020.
53. Kelly Miller and Hal Alper, 2019, *Yarrowia lipolytica: More Than an Oleaginous Workhorse*, **Applied Microbiology and Biotechnology**, 103(23-24), 9251–9262, December 2019.
54. Ruiqi Tang, Peiliang Ye, Hal Alper, Zhanying Liu, Xinqing Zhao, and Fengwu Bai, *Identification and characterization of novel xylose isomerases from a Bos taurus fecal metagenome*, **Applied Microbiology and Biotechnology**, 103(23-24) 9465–9477, December 2019.
55. James Wagner, Kevin Reed, Simon d'Oelsnitz, Joshua Wiggers, and Hal Alper, *Improving Ionic Liquid Tolerance in Saccharomyces cerevisiae Through Heterologous Expression and Directed Evolution of an ILT1 Homolog from Yarrowia lipolytica*, **Journal of Industrial Microbiology and Biotechnology**, 46(12), 1715–1724, December 2019.
56. Matthew Deaner and Hal Alper, *Enhanced scale and scope of genome engineering and regulation using CRISPR/Cas in Saccharomyces cerevisiae*, **FEMS Yeast Research**, 19(7), foz076, November 2019.
57. Cory Schwartz, Jan-Fang Cheng, Robert Evans, Christopher Schwartz, James Wagner, Scott Anglin, Adam Beitz, Weihua Pan, Stefano Lonardi, Mark Blenner, Hal Alper, Yasuo Yoshikuni, and Ian Wheeldon, *Validating genome-wide CRISPR-Cas9 function improves screening in the oleaginous yeast Yarrowia lipolytica*, **Metabolic Engineering**, 55, 102-110, September 2019.
58. Joseph Cheng, Nicholas Morse, James Wagner, Scott Tucker, and Hal Alper, *Design and evaluation of synthetic terminators for regulating mammalian cell transgene expression*, **ACS Synthetic Biology**, 8(6), 1263-1275, June 2019.
59. Kristin Presnell and Hal Alper, *Systems Metabolic Engineering Meets Machine Learning: A new era for data-driven metabolic engineering*, **Biotechnology Journal**, 14(9), 1800416, September 2019.
60. Kristin Presnell, Madeleine Flexer-Harrison, and Hal Alper, *Design and synthesis of synthetic UP elements for modulation of gene expression in Escherichia coli*, **Synthetic and Systems Biotechnology**, 4(2), 99–106, June 2019.
61. Jyun-Liang Lin, Holly Ekas, Matthew Deaner, and Hal Alper, *CRISPR-PIN: Modifying gene position in the nucleus via dCas9-mediated tethering*, **Synthetic and Systems Biotechnology**, 4(2), 73-78, June 2019.
62. Holly Ekas, Matthew Deaner, and Hal Alper, *Recent advancements in fungal-derived fuel and chemical production and commercialization*, **Current Opinion in Biotechnology**, 57, 1-9, June 2019.
63. Shuo-Fu Yuan and Hal Alper, *Metabolic engineering of microbial cell factories for production of nutraceuticals*, **Microbial Cell Factories**, 18:46, March 2019.

64. Shenghu Zhou and Hal Alper, *Strategies for directed and adapted evolution as part of microbial strain engineering*, **Journal of Chemical Technology and Biotechnology**, 94(2), 366-376, February 2019.
65. Claire Palmer and Hal Alper, *Expanding the chemical palette of industrial microbes: metabolic engineering for type III PKS-derived polyketides*, **Biotechnology Journal**, 14(1), 1700463, January 2019.
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B. Book Chapters

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C. Patents and Patent Applications

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17. Gregory Stephanopoulos, Hal Alper, and Yong-Su Jin, Gene targets for enhanced carotenoids production. US Patent Number 7,695,932 B2. Patent Issued: April 13, 2010.

D. Presentations/Invited Talks

1. Hal Alper, Invited Speaker, *Engineering enzymes for the depolymerization of plastics including PET*, 46th Symposium on Biomaterials, Fuels and Chemicals, Alexandria, VA, April 30, 2024.
2. Hal Alper, Invited Speaker, *Beyond the test-tube: metabolic engineering using microdroplets and hydrogels*, University of Minnesota, Department of Chemical Engineering, Minneapolis, Minnesota, January 25, 2024.
3. Hal Alper, Invited Speaker, *Engineering biology to develop “a taste for waste”*, Charles W. Davidson College of Engineering Interdisciplinary Speaker Series, San Jose State University, San Jose, CA, October 13, 2023.
4. Hal Alper, Invited Speaker, *Metabolic Engineering using Microdroplets*, Metabolic Engineering 15 Conference, Singapore, June 12, 2023.
5. Hal Alper, John van Geuns Invited Lecturer, *Machine learning-aided engineering of PET degrading enzymes*, University of Amsterdam AI4 Enzyme Instructed Catalysts Workshop, Amsterdam, Netherlands, May 15, 2023.
6. Hal Alper, Invited Speaker, *Can plastic-eating enzymes save our planet?*, South by Southwest Festival—Climate Change Track, Austin, Texas, March 11, 2023.

7. Hal Alper, Invited Speaker, *Beyond the test-tube: metabolic engineering using microdroplets and hydrogels*, Chalmers University of Technology, Gothenburg, Sweden, February 23, 2023.
8. Hal Alper, Invited Speaker, *Machine learning-aided engineering of PET degrading enzymes*, World PET Biorecycling Summit, Paris, France, December 7, 2022.
9. Hal Alper, Invited Speaker, *Engineering Plastic-eating Enzymes: A case study in breaking down environmentally-throttling PET plastics*, Frontiers of Science session in Council of Scientific Society Presidents (CSSP) Virtual Leadership Workshop, December 5, 2022.
10. Hal Alper, Invited Speaker, *The power of biology to build-up and break-down polymeric materials*, Material Research Society Fall Meeting, Boston, MA, November 30, 2022.
11. Hal Alper, Invited Speaker, *Modern bioengineering and its home in chemical engineering*, American Institute for Chemical Engineers Annual Meeting, Phoenix, AZ, November 15, 2022.
12. Hal Alper, Invited Speaker, *Machine learning-aided engineering of PET degrading enzymes*, Molecular Biotechnology Training Program, NC State University, Presented Online, November 9, 2022.
13. Hal Alper, Invited Speaker, *Metabolic Engineering and Synthetic Biology for a More Sustainable Future*, University of Washington, Department of Chemistry, October 13, 2022.
14. Hal Alper, Invited Speaker, *Machine learning-aided engineering of PET degrading enzymes*, British Plastics Federation Online Seminar, Online, October 11, 2022.
15. Hal Alper, Invited Speaker, *Machine learning-aided engineering of PET degrading enzymes*, Plastics Symposium, Pasadena, TX, September 9, 2022.
16. Hal Alper, Invited Speaker, *Beyond the test-tube: metabolic engineering using microdroplets and hydrogels*, Amyris Corporation, Emeryville, CA, August 10, 2022.
17. Hal Alper, Invited Speaker, *Engineering biology to develop “a taste for waste”*, Society for Industrial Microbiology and Biotechnology Annual Meeting, San Francisco, CA August 9, 2022.
18. Hal Alper, Invited Speaker, *Metabolic Engineering and Synthetic Biology for a More Sustainable Future*, New England Biolabs, Ipswich, MA, August 4, 2022.
19. Hal Alper, Invited Speaker, *Metabolic Engineering and Synthetic Biology for a More Sustainable Future*, iCanX Seminar Speaker, Virtual Seminar, June 10, 2022.
20. Hal Alper, Invited Speaker, *Microdroplet-enabled Metabolic Engineering and Directed Evolution*, 44th Symposium on Biomaterials, Fuels and Chemicals, New Orleans, LA, May 2, 2022.
21. Hal Alper, Invited Speaker, *Beyond the test-tube: metabolic engineering using microdroplets and hydrogels*, Northeastern University, China, Virtual Seminar, April 21, 2022.
22. Hal Alper, Keynote Speaker, *Metabolic Engineering and Synthetic Biology for a More Sustainable Future*, 2022 Korean Society for Biotechnology and Bioengineering (KSSB) Spring Meeting and International Symposium, Virtual Conference Attendance, April 13, 2022.

23. Hal Alper, Invited Speaker, *Beyond the test-tube: metabolic engineering using microdroplets and hydrogels*, Tufts University, Department of Chemical Engineering Seminar Series, Virtual Seminar, November 1, 2021.
24. Hal Alper, Invited Speaker, *Beyond the test-tube: metabolic engineering using microdroplets and hydrogels*, Joint BioEnergy Institute Seminar Series, Online, October 26, 2021.
25. Hal Alper, Invited Speaker, *Optimizing pathways in yeast using a CRISPR-dCas9-based approach*, 4th International Conference on CRISPR Technologies, Online, October 1, 2021.
26. Hal Alper, Invited Speaker, *Engineering an expanded chemical palette in cells*, University of Tennessee, Department of Chemical Engineering Seminar Series, Virtual Seminar, September 21, 2021.
27. Hal Alper, Invited Speaker, *An integrated cell/cell-free metabolic engineering approach for rapid prototyping of genes and pathways*, 15th Annual Genomics of Energy & Environment Meeting, Online, September 1, 2021.
28. Hal Alper, Invited Speaker, *Combining cells and hydrogels for on-demand production capabilities*, Engineered Living Materials Conference, Online, May 4, 2021.
29. Hal Alper, Invited Speaker, *Directed evolution and selection approaches in yeast for ESPS and beyond*, Bayer Corporation, Online, April 20, 2021.
30. Hal Alper, Invited Speaker, *Microdroplet-enabled Metabolic Engineering and Directed Evolution*, International Union of Microbiological Societies Congresses, Online, November 16-20, 2020.
31. Hal Alper, Invited Speaker, *Enhanced scale and scope of genome engineering and regulation using CRISPR/Cas in *Saccharomyces cerevisiae**, FEMS Yeast Research Webinar, Online, November 5, 2020.
32. Hal Alper, Invited Speaker, *Engaging with JGI via DNA Synthesis Grants: Guide RNAs for gene activation/ suppression in microbes*, Joint Genome Institute Engagement Webinar, Online, July 8, 2020.
33. Hal Alper, Invited Speaker, *Engineering an expanded chemical palette in cells*, Tsinghua University Online Forum of Industrial Biocatalysis, Online, April 22, 2020.
34. Hal Alper, Invited Speaker, *Engineering an expanded chemical palette in cells*, Rice University, Department of Chemical Engineering, Houston, Texas, February 6, 2020.
35. Hal Alper, Invited Speaker, *Engineering an expanded chemical palette in cells*, Kobe University, Graduate School of Science, Technology and Innovation, Kobe, Japan, January 15, 2020.
36. Hal Alper, Plenary Lecture, Division 15c, *Microdroplet-enabled Metabolic Engineering and Directed Evolution*, American Institute for Chemical Engineers Annual Meeting, Orlando, FL, November 12, 2019.
37. Hal Alper, Keynote Lecture, *Metabolic Engineering of *Yarrowia lipolytica* for fuels and chemicals production*, International Conference on Green Biomanufacturing (ICGB 2019), Beijing, China, October 24, 2019.
38. Hal Alper, Invited Speaker, *Microdroplet-enabled Metabolic Engineering and Directed Evolution*, Metabolic Engineering Summit 2019, Tianjin, China, October 22, 2019.
39. Hal Alper, Invited Speaker, *Engineering an expanded chemical palette in cells*, Fudan University, College of Life Science, Shanghai, China, August 16, 2019.

40. Hal Alper, Invited Speaker, *Engineering an expanded chemical palette in cells*, Institute for Microbiology, Chinese Academy of Sciences, Beijing, China, August 14, 2019.
41. Hal Alper, Invited Speaker, *Engineering an expanded chemical palette in cells*, Beijing Institute of Technology, Department of Chemical Engineering, Beijing, China, August 13, 2019.
42. Hal Alper, Invited Speaker, *Metabolic Engineering of Yarrowia lipolytica for fuels and chemicals production*, Society for Microbiology and Biotechnology Annual Meeting, Washington, DC, July 24, 2019.
43. Hal Alper, Keynote Speaker, *Metabolic Engineering Meets Evolution: New approaches for the optimization and selection of metabolic phenotypes*, 14th Asian Conference on Biotechnology, Taipei, Taiwan, July 3, 2019.
44. Hal Alper, Invited Speaker, *Optimization, Evolution, and Control of Pathways and Metabolism in Yeast*, JGI User Meeting Symposium, San Francisco, CA, April 2, 2019.
45. Hal Alper, Invited Speaker, *Optimization, Evolution, and Control of Pathways and Metabolism in Yeast*, 9th International Conference on Biomolecular Engineering, Newport Beach, CA, January 7, 2019.
46. Hal Alper, Invited Speaker, *Engineering an expanded chemical palette in cells*, School of Life Science and Biotechnology, Shanghai Jiao Tong University, Shanghai, China, October 19, 2018.
47. Hal Alper, Invited Speaker, *Optimization, Evolution, and Control of Pathways and Metabolism in Yeast*, Nature Conference on Synthetic Biology: Resource Allocation in Natural and Unnatural Systems, Shanghai, China, October 16, 2018.
48. Hal Alper, Invited Speaker, *Engineering an expanded chemical palette in cells*, American Society for Microbiology Conference for Undergraduate Educators, Austin, TX, July 27, 2018.
49. Hal Alper, Invited Presentation, *Metabolic engineering and synthetic biology in the host Yarrowia lipolytica*, Manus Corporation, Cambridge, MA, July 25, 2018.
50. Claire Palmer, Kelly Markham, and Hal Alper, Poster Presentation, *Rewiring Yarrowia lipolytica for polyketide production*, Metabolic Engineering 12 Conference, Munich, Germany, June 25, 2018.
51. James Wagner, Leqian Liu, Joseph Abatemarco, Maen Sarhan, Aaron Lin, Shuo-Fu Yuan, Maya Venkataraman, Adam Abate, and Hal Alper, Poster Presentation, *High Throughput, Microdroplet-Assisted Evolution of Secretory Phenotypes*, Metabolic Engineering 12 Conference, Munich, Germany, June 25, 2018.
52. Lauren Cordova and Hal Alper, Poster Presentation, *Rewiring lipid compositions in Yarrowia lipolytica for α -linolenic acid production*, Metabolic Engineering 12 Conference, Munich, Germany, June 25, 2018.
53. Hal Alper, Invited Speaker, *Metabolic Engineering Meets Evolution: New approaches for the optimization and selection of metabolic phenotypes*, Metabolic Engineering 12 Conference, Munich, Germany, June 25, 2018.
54. Hal Alper, Invited Speaker, *Engineering an expanded chemical palette in cells*, Institute of Food Science and Technology CAAS, Beijing, China, May 25, 2018.
55. Hal Alper, Invited Speaker, *Engineering an expanded chemical palette in cells*, School of Life Sciences, Tsinghua University, Beijing, China, May 24, 2018.

56. Hal Alper, Zhang Dayu Young Investigator Lecture, *Engineering an expanded chemical palette in cells*, Dalian Institute of Chemical Physics, Dalian, China, May 23, 2018.
57. Hal Alper, Keynote Lecture, *Optimization, Evolution, and Control of Pathways and Metabolism in Yeast*, American Chemical Society National Meeting, New Orleans, LA, March 21, 2018.
58. Hal Alper, Invited Speaker, *Engineering an expanded chemical palette in cells*, Department of Chemical and Environmental Engineering, University of California-Riverside, Riverside, CA, February 16, 2018.
59. Hal Alper, Invited Speaker, *Optimization, Evolution, and Control of Pathways and Metabolism in Yeasts*, 9th International Symposium of Innovative BioProduction Kobe (iBioK), University of Kobe, Kobe, Japan, February 2, 2018.
60. Hal Alper, Invited Speaker, *Engineering an expanded chemical palette in cells*, Department of Chemical Engineering, University of Delaware, Newark, DE, October 20, 2017.
61. Hal Alper, Invited Speaker, *Synthetic Biology and Directed Evolution Approaches in Yeasts*, Novozymes, Franklinton, NC, October 5, 2017.
62. Shuo-Fu Yuan, James Wagner, Joseph Abatemarco, Maen Sarhan, Jyun-Liang Lin Lin, Leqian Liu, Adam Abate and Hal Alper, Poster Presentation, *RNA-Aptamer-In-Droplet (RAPID) High Throughput Screening for Secretory Phenotypes*, DNA 23 Conference, Austin, TX, September 25, 2017.
63. Hal Alper, Invited Speaker, *Synthetic Biology and Metabolic Engineering of Yeasts*, Zymergen Corporation, Emeryville, CA, September 12, 2017.
64. Claire Palmer, Kelly Markham, and Hal Alper, *Engineering Yarrowia lipolytica for triacetic acid lactone production*, SIMB National Meeting, Denver, CO, July 31, 2017.
65. Hal Alper, Invited Speaker, *Synthetic Biology and Evolutionary Approaches for Genes and Pathways in Yeast*, Monsanto Corporation, St. Louis, MO, July 26, 2017.
66. Hal Alper, Invited Speaker, *Engineering an expanded chemical palette in cells*, A*STAR Research Institute, Biopolis, Singapore, July 20, 2017.
67. Hal Alper, Plenary Speaker, *Synthetic Biology and Metabolic Engineering of Yeasts for Chemicals and Fuels Production*, 15th International Congress of Mycology and Eukaryotic Microbiology, Singapore, July 19, 2017.
68. Hal Alper, Invited Speaker, *Engineering an expanded chemical palette in cells*, NUS Synthetic Biology for Clinical and Technological Innovation (SynCTI) center, National University of Singapore, Singapore, July 18, 2017.
69. John Lin, Lauren Cordova, Hal Alper, and Livia Eberlin, Poster Presentation, Lipid Characterization in Genetically Engineered *Yarrowia lipolytica* by Desorption Electrospray Ionization Mass Spectrometry, Poster Presentation, 65th American Society for Mass Spectroscopy, Indianapolis, IN, June 4, 2017.
70. Hal Alper, Invited Plenary and Award Speaker, *Engineering an expanded chemical palette in cells*, 11th Panhellenic Scientific Conference in Chemical Engineering, Thessaloniki, Greece, May 25, 2017.
71. Hal Alper, Invited Plenary Speaker, *Metabolic Engineering of Yarrowia lipolytica for Chemicals Production*, 7th International Forum on Industrial Bioprocessing, Wuxi, China, May 21, 2017.

72. Hal Alper, Invited Speaker, *Directed and Adaptive Evolution Approaches for Strain Engineering*, School of Biotechnology, Jiangnan University, Wuxi, China, May 20, 2017.
73. Hal Alper, Keynote Speaker, *Synthetic control of transcription: a decade's perspective on synthetic promoter design*, Academy of Finland's Synthetic Biology Final Programme, Helsinki, Finland, April 26, 2017.
74. Hal Alper, Invited Speaker, *Engineering an expanded chemical palette in cells*, Department of Chemical Engineering, Northwestern University, Evanston, IL, March 30, 2017.
75. Hal Alper, Invited Speaker, *Engineering an expanded chemical palette in cells*, Department of Chemical Engineering, Clemson University, Clemson, NC, March 16, 2017.
76. Hal Alper, Invited Speaker, *Synthetic Biology and Directed Evolution Approaches in Yeasts*, BASF, San Diego, CA, February 17, 2017.
77. Nathan Crook, Jie Sun, Alexander Schmitz, and Hal Alper, *Optimization of a Tunable RNAi Screening Approach for Systems Engineering of Complex Phenotypes in Yeast*, American Institute of Chemical Engineers Annual meeting, San Francisco, CA, November 15, 2016
78. Nicholas Morse and Hal Alper, *Library and Model Derived Synthetic Terminators for Yeast*, American Institute of Chemical Engineers Annual meeting, San Francisco, CA, November 15, 2016
79. Matthew Deaner and Hal Alper, *A Generalizable Crispr-Cas9 System for Rapid and Scalable Gene Target Discovery and Optimization of Metabolism*, American Institute of Chemical Engineers Annual meeting, San Francisco, CA, November 15, 2016
80. Hal Alper, Invited Speaker, *Synthetic Biology and Metabolic Engineering of Yeasts*, Shanghai Jiao Tong University, Shanghai, China, October 21, 2016
81. Hal Alper, Invited Speaker, *Metabolic engineering of yeasts for advanced fuels and chemicals*, International Conference on Metabolic Science, Shanghai, China, October 21, 2016
82. Hal Alper, Invited Speaker, *Synthetic Biology and Metabolic Engineering of Yeasts*, Jiangnan University, Wuxi, China, October 20, 2016
83. Hal Alper, Invited Keynote Speaker, *Metabolic Engineering of Yarrowia lipolytica for fuels and chemicals production*, 13th Genetics of Industrial Microorganisms Conference, Wuhan, China, October 19, 2016.
84. Hal Alper, Invited Speaker, *Directed and Adaptive Evolution Approaches for Strain Engineering*, Society of Industrial Microbiology and Biotechnology Annual Meeting, New Orleans, LA, July 26, 2016.
85. Hal Alper, *In Vivo continuous Evolution of Genes and Pathways in Yeast*, Synthetic Biology: Engineering, Evolution & Design (SEED) conference, Chicago, IL, July 21, 2016.
86. Nicholas Morse and Hal Alper, Poster Presentation, *Library and Model Derived Synthetic Terminators for Yeast*, Synthetic Biology: Engineering, Evolution & Design (SEED) conference, Chicago, IL, July 21, 2016.
87. Hal Alper, Invited Speaker, *Optimization and Evolution of Pathways and Metabolism in Yeast*, Metabolic Engineering XI Conference, Kobe, Japan, June 29, 2016.

88. Kelly Markham and Hal Alper, Poster Presentation, *Expanding the Substrate Range and Product Portfolio of Y. lipolytica through Metabolic Pathway Engineering and Synthetic Biology Tool Development*, Metabolic Engineering XI Conference, Kobe, Japan, June 29, 2016.
89. Hal Alper, Invited Speaker, *Synthetic Biology and Metabolic Engineering of Yeasts for Chemicals and Fuels Production*, Center for Biosustainability (CFB) Seminar Series, May 17, 2016.
90. Hal Alper, Invited Speaker, *Synthetic Biology and Metabolic engineering of yeasts chemicals production*, Dow AgroSciences, Indianapolis, IN, April 11, 2016.
91. Hal Alper, Invited Speaker, *Engineering an expanded chemical palette in cells*, Department of Chemical Engineering, Princeton University, Princeton, NJ, March 9, 2016.
92. Hal Alper, Invited Speaker in Plenary Session, *Synthetic control of transcription: a decade's perspective on synthetic promoter design*, American Institute of Chemical Engineers Annual meeting, Salt Lake City, UT, November 10, 2015.
93. Hal Alper, *Metabolic Engineering through Evolution*, American Institute of Chemical Engineers Annual meeting, Salt Lake City, UT, November 9, 2015.
94. Hal Alper, Invited Speaker, *Synthetic Biology and Metabolic Engineering of Yeasts*, BASF, Tarrytown, NY, October 23, 2015.
95. Hal Alper, Invited Speaker—Mellichamp Lecture, *Engineering an expanded chemical palette in cells*, Department of Chemical Engineering, Purdue University, West Lafayette, IN, October 6, 2015.
96. Hal Alper, Invited Speaker, *Synthetic Biology Paradigms for Cell Line Development*, Bristol-Myers-Squibb, Danvers, MA, September 28, 2015.
97. Hal Alper, Invited Speaker, *Metabolic Engineering of Yarrowia lipolytica for fuels and chemicals production*, 2015 Metabolic Engineering and Green Manufacturing Symposium, Beijing, China, July 11, 2015.
98. Hal Alper, Invited Speaker, *Metabolic engineering of yeasts for fuels and chemicals*, Chinese Academy of Sciences, Institute of Microbiology, Beijing, China, July 9, 2015.
99. Hal Alper, Invited Speaker, *Metabolic engineering of yeasts for fuels and chemicals*, Tianjin University, Department of Chemical Engineering, Tianjin, China, July 8, 2015.
100. Hal Alper, Invited Speaker, *Metabolic engineering of yeasts for fuels and chemicals*, Tianjin Institute for Industrial Biotechnology, Beijing, China, July 7, 2015.
101. Hal Alper, Invited Speaker, *Synthetic Control of Transcription in Cells*, 18th Annual American Society of Gene & Cell Therapy Conference, New Orleans, LA, May 13, 2015.
102. Hal Alper, Invited Speaker, *Synthetic Biology and Metabolic Engineering of Yeasts*, Energy Biosciences Institute, University of California, Berkeley, Berkeley, CA, March 13, 2015.
103. Hal Alper, Invited Speaker, *Synthetic Biology and Metabolic Engineering of Yeasts*, Zymergen Corporation, Emeryville, CA, March 12, 2015.
104. Hal Alper, Invited Speaker, *Synthetic Biology and Metabolic Engineering of Yeasts*, Intrexon Corporation, San Francisco, CA, March 11, 2015.
105. Hal Alper, Invited Speaker, *Synthetic Biology and Metabolic Engineering of Yeasts*, REG Life Sciences, LLC, San Francisco, CA, March 11, 2015.

106. Hal Alper, Invited Speaker, *A Condition-Specific Codon Optimization Approach for Improved Heterologous*, Gene Expression, Engineering Genes, Vectors, Constructs and Clones at Cambridge Healthtech Institute's 14th Annual PepTalk, San Diego, CA, January 19, 2015.
107. Heidi Redden and Hal Alper, *Design and Characterization of Synthetic, Minimal Promoters in Yeast*, 5th International Conference on Biomolecular Engineering, Lost Pines, TX, January 12, 2015.
108. Jie Sun, Nathan Crook, Joe Abatamarco, and Hal Alper, *A Novel, In Vivo Continuous Evolution (ICE) Strategy in Saccharomyces cerevisiae to Create Pathway and Protein Variants*, 5th International Conference on Biomolecular Engineering, Lost Pines, TX, January 12, 2015.
109. Andrew Hill, John Blazeck, Mariam Jamoussi, Jarrett Miller, and Hal Alper, *Metabolic Engineering of Yarrowia Lipolytica for the Production of Itaconic Acid*, American Institute of Chemical Engineers Annual meeting, Atlanta, GA, November 20, 2014.
110. Andrew Hill, Kelly Markham, Rebecca Knight, Jarrett Miller, Mariam Jamoussi and Hal Alper, *Engineering Yarrowia Lipolytica As a Platform for the Biomodification of Native Lipids into Diverse Oleochemicals*, American Institute of Chemical Engineers Annual meeting, Atlanta, GA, November 19, 2014.
111. Joe Abatamarco, Nathan Crook, Jie Sun and Hal Alper, *Rapid Directed Evolution of Enzymes and Regulatory Factors through an in Vivo Continuous Evolution (ICE) Approach in Yeast*, American Institute of Chemical Engineers Annual meeting, Atlanta, GA, November 17, 2014.
112. Hal Alper, Invited Speaker, *Synthetic Biology and Directed Evolution Strategies for Metabolic Engineering*, Interdepartmental Genetics (IG) Workshop at Iowa State University, Ames, IA, October 14, 2014.
113. Hal Alper, Invited Speaker, *Metabolic Engineering of Yeasts for Fuels and Chemicals*, DSM Nutritional Products Microbia, Inc., Lexington, MA, May 9, 2014.
114. Kathleen Curran, Nathan Crook, Ashty Karim, Akash Gupta, Allison Wagman, and Hal Alper, *Model-based design of synthetic yeast promoters via tuning of nucleosome architecture*, American Chemical Society Annual Meeting, Dallas, TX, March 18, 2014.
115. Nathan Crook, Joeseeph Abatamarco, Jie Sun, Alexander Schmitz, and Hal Alper, *Rapid evolution of parts and pathways through an in vivo continuous evolution (ICE) approach in yeast*, American Chemical Society Annual Meeting, Dallas, TX, March 17, 2014.
116. Andrew Hill, Rebecca Knight, Jarrett Miller, Mariam Jamoussi, and Hal Alper, *Rewiring Yarrowia lipolytica's lipogenesis for the production of diverse oleochemicals*, American Chemical Society Annual Meeting, Dallas, TX, March 16, 2014.
117. Hal Alper, Invited Speaker, *Using Yeasts to Produce Chemicals*, School of Chemical & Biomolecular Engineering, Georgia Institute of Technology, Atlanta, GA, February 19, 2014.
118. Hal Alper, Invited Speaker, *Metabolic Engineering of Yarrowia lipolytica for fuels and chemicals applications*, 5th International Symposium of Innovative BioProduction Kobe (iBioK), University of Kobe, Kobe, Japan, January 30, 2014.
119. Hal Alper, Invited Speaker, *Using Yeasts to Produce Chemicals*, Mitsubishi Chemical Company, Yokohama, Japan, January 28, 2014.

120. Nathan Crook, Alexander Schmitz, and Hal Alper, *Facile Generation of Knockdown Phenotypes in Yeast Through RNA Interference*, American Institute of Chemical Engineers Annual meeting, San Francisco, CA, November 8, 2013.
121. Sun-mi Lee, Taylor Jellison, and Hal Alper, *Combinatorial and Evolutionary Engineering of Pentose Sugar Catabolism in Saccharomyces Cerevisiae for Efficient Biofuels Production*, American Institute of Chemical Engineers Annual meeting, San Francisco, CA, November 8, 2013.
122. Nathan Crook, Joe Abatemarco, Jie Sun, Alexander Schmitz, and Hal Alper, *Rapid Evolution of Enzymes and Regulatory Factors Through An in Vivo Continuous Evolution (ICE) Approach in Yeast*, American Institute of Chemical Engineers Annual meeting, San Francisco, CA, November 7, 2013.
123. Eric Young, Alice Tong, and Hal Alper, Poster Presentation, *Rewiring Yeast Sugar Transporter Preference Through Modifying a Conserved Protein Motif*, American Institute of Chemical Engineers Annual meeting, San Francisco, CA, November 6, 2013.
124. Kathleen Curran, Nathan Crook, Ashty Karim, Akash Gupta, and Hal Alper, *Rational Design of Synthetic Yeast Promoters Using Systems-Level Bioinformatics Models*, American Institute of Chemical Engineers Annual meeting, San Francisco, CA, November 5, 2013.
125. Anny Pan and Hal Alper, Poster Presentation, *Metabolic Engineering of the Yeast Saccharomyces Cerevisiae for Itaconic Acid Production*, American Institute of Chemical Engineers Annual meeting, San Francisco, CA, November 4, 2013.
126. Leqian Liu, Andrew Hill, John Blazeck, and Hal Alper, *Engineering Yarrowia Lipolytica for Fuels and Oleochemicals Applications*, American Institute of Chemical Engineers Annual meeting, San Francisco, CA, November 4, 2013.
127. Kathleen Curran, Ashty Karim, Akash Gupta, and Hal Alper, *Identification of High Capacity Terminators for Improved Control of Gene Expression and Metabolic Engineering in Saccharomyces cerevisiae*, American Institute of Chemical Engineers Annual meeting, San Francisco, CA, November 3, 2013.
128. Hal Alper, Invited Speaker, *Synthetic control of transcription: from hybrid promoters to promoter engineering to synthetic operon design*, SB6.0: the Sixth International Meeting on Synthetic Biology, London, England, July 10, 2013.
129. Hal Alper, Invited Speaker, *Directed evolution strategies for cellular and metabolic engineering*, Department of Chemical Engineering, Tsinghua University, Beijing, China, June 21, 2013.
130. Hal Alper, Invited Speaker, *Synthetic control of transcription: from hybrid promoters to promoter engineering to synthetic operon design*, ECI Biochemical and Molecular Engineering XVIII Conference, Beijing, China, June 17, 2013.
131. Hal Alper, Invited Speaker, “*Metabolic Engineering of Yarrowia lipolytica for fuels and chemicals applications*”, 3rd Copenhagen Bioscience Conference on ‘Cell Factories and Biosustainability, Copenhagen, Denmark, May 7, 2013.
132. Hal Alper, Invited Speaker, “*Synthetic Promoter Engineering*”, American Chemical Society Annual Meeting, New Orleans, LA, April 9, 2013.
133. Hal Alper, Invited Speaker, “*Directed Evolution Strategies for Metabolic Engineering*”, Department of Chemical Engineering, Pennsylvania State University, College Station, PA, March 14, 2013.

134. Hal Alper, Invited Speaker, “*Directed Evolution and Synthetic Biology Strategies for Metabolic Engineering*”, Department of Chemical Engineering, California Institute of Technology, Pasadena, CA, February 7, 2013.
135. Hal Alper, Invited Speaker, “*Pathway Engineering in Yeast via Directed Evolution*”, International Conference on Biomolecular Engineering, Fort Lauderdale, FL, January 15, 2013.
136. Hal Alper, Invited Speaker, “*Constructs and Methodologies for High-level Transgene Expression in Human Cell Lines*”, Shire Pharmaceuticals, Lexington, Massachusetts, December 19, 2012.
137. Hal Alper, Invited Speaker, “*Synthetic control of transcription: from hybrid promoters to promoter engineering to synthetic operon design*”, Synthetic Biology Cold Spring Harbor Asia Conference, Suzhou, China, November 28, 2012.
138. Hal Alper, Invited Speaker, “*Directed evolution strategies for cellular and metabolic engineering*”, Dept. Microbiology and Cell Science, University of Florida at Gainesville, Gainesville, FL, November 16, 2012.
139. John Blazeck and Hal Alper, “*Enabling Transcriptional Control in Saccharomyces Cerevisiae Utilizing a Synthetic Hybrid Promoter Engineering Approach*”, American Institute of Chemical Engineers Annual meeting, Pittsburgh, PA, October 31, 2012.
140. John Blazeck and Hal Alper, “*Engineering a Yeast Conversion System for the Novel Production of Alkanes*”, American Institute of Chemical Engineers Annual meeting, Pittsburgh, PA, October 31, 2012.
141. Eric Young, Austin Comer, and Hal Alper, “*Engineering Exogenous Sugar Catabolism in Saccharomyces Cerevisiae for Improved Biofuels Production*”, American Institute of Chemical Engineers Annual meeting, Pittsburgh, PA, October 30, 2012.
142. Hal Alper, Invited Speaker—Distinguished Alumni Speaker, “*Directed Evolution and Synthetic Biology Strategies for Metabolic Engineering*”, Department of Chemical Engineering, University of Maryland at College Park, College Park, MD, October 19, 2012.
143. Hal Alper, Invited Speaker, “*Directed Evolution and Synthetic Biology Strategies for Metabolic Engineering*”, Biotechnology Institute, University of Minnesota, Minneapolis, MN, October 11, 2012.
144. Hal Alper, Invited Speaker, “*Directed Evolution and Synthetic Biology Strategies for Metabolic Engineering*”, E. I. du Pont de Nemours and Company, Wilmington, DE, October 5, 2012.
145. Hal Alper, Invited Speaker, “*Directed Evolution and Synthetic Biology Strategies for Metabolic Engineering*”, Department of Chemical Engineering, Massachusetts Institute of Technology, Cambridge, MA, September 14, 2012.
146. Hal Alper, John Blazeck, Kathleen Curran, and Nathan Crook, “*Synthetic control of transcription: from hybrid promoters to promoter engineering to synthetic operon design*”, Society of Industrial Microbiology and Biotechnology Annual Meeting, Washington, DC, August 15, 2012.
147. Kathleen Curran, John Leavitt, Ashty Karim, Hal Alper, “*Metabolic Engineering of Muconic Acid Production in Saccharomyces cerevisiae*”, Society of Industrial Microbiology and Biotechnology Annual Meeting, Washington, DC, August 14, 2012.

148. Hal Alper, Eric Young, and Sun-mi Lee, Invited Talk, “*Application of directed evolution of xylose transport and catabolism in yeast*”, Society of Industrial Microbiology and Biotechnology Annual Meeting, Washington, DC, August 13, 2012.
149. Hal Alper, Invited Talk, “*Directed evolution and Synthetic Biology Strategies for Cellular and Metabolic Engineering*”, Amyris, Inc., Emeryville, CA, August 8, 2012.
150. Hal Alper, Invited Talk, “*Directed evolution and Synthetic Biology Strategies for Cellular and Metabolic Engineering*”, Gevo, Inc., Denver, CO, August 2, 2012.
151. Hal Alper, John Blazeck, Kathleen Curran, and Nathan Crook, “*Synthetic control of transcription: from hybrid promoters to promoter engineering to synthetic operon design*”, ECI Metabolic Engineering IX, Biarritz, France, Oral Presentation, June 6, 2012.
152. Hal Alper and Eric Young, “*A molecular transporter engineering approach to improving xylose catabolism in Saccharomyces cerevisiae*”, ECI Metabolic Engineering IX, Biarritz, France, Poster Presentation, June 3-7, 2012.
153. Amanda Lanza, John Blazeck, Nathan Crook, and Hal Alper, “*A quantitative, graded dominant mutant approach for probing protein function and gene regulation*”, ECI Metabolic Engineering IX, Biarritz, France, Poster Presentation, June 3-7, 2012.
154. Hal Alper, Invited Talk, “*Synthetic Biology and Metabolic Engineering for Fuels, Chemicals, and Biopharmaceuticals Production*”, Life Technologies Corporation, Carlsbad, California, May 8, 2012.
155. Hal Alper, Invited Talk, “*Directed evolution strategies for cellular and metabolic engineering*”, Department of Chemical Engineering, The University of California, Los Angeles, May 4, 2012.
156. Hal Alper, Invited Talk, “*Directed evolution strategies for cellular and metabolic engineering*”, Department of Chemical Engineering, University of Southern California, May 3, 2012.
157. Hal Alper, Poster Presentation, “*Constructs and Methodologies for High-Level Transgene Expression*”, ECI Cell Culture and Engineering XII Conference, Scottsdale, Arizona, April 22-27.
158. Hal Alper, Eric Young, and Sun-mi Lee, “*Application of directed evolution of xylose transport and catabolism in yeast*”, American Chemical Society Annual Meeting, San Diego, CA, March 26, 2012.
159. Hal Alper, Amanda Lanza, John Blazeck, and Nathan Crook, “*Linking Genotype and Phenotype Through a Graded Dominant Mutant Approach*”, American Chemical Society Annual Meeting, San Diego, CA, March 25, 2012.
160. Hal Alper, Invited Talk, “*Re-engineering multicloning sites for function and convenience*”, Engineering Genes, Vectors, Constructs and Clones at Cambridge Healthtech Institute’s 11th Annual PepTalk, San Diego, CA, January 9, 2012.
161. Hal Alper, Invited Talk, “*Cellular and Pathway Engineering via Directed Evolution: Toward an integrative metabolic engineering approach*”, Department of Chemical Engineering, The University of Illinois-Urbana Champaign, December 6, 2011.
162. Amanda Lanza, John Blazeck, Nathan Crook, and Hal Alper, “*Linking Genotype and Phenotype Through a Graded Dominant Mutant Phenotype Approach*” American Institute of Chemical Engineers Annual meeting, Minneapolis, MN, October 20, 2011.

163. Eric Young and Hal Alper, “*Improving Exogenous Sugar Utilization by Engineered Transporters*” American Institute of Chemical Engineers Annual meeting, Minneapolis, MN, October 19, 2011.
164. Nathan Crook, Elizabeth Freeman, and Hal Alper, “*Re-Engineering Multicloning Sites for Function and Convenience*” American Institute of Chemical Engineers Annual meeting, Minneapolis, MN, October 19, 2011.
165. Amanda Lanza and Hal Alper, “*Exploring the Cre/Lox System for Targeted Integration Into the Human Genome*”, American Institute of Chemical Engineers Annual meeting, Minneapolis, MN, October 17, 2011.
166. Hal Alper, Invited Talk, “*Using Metabolic Engineering and Protein Engineering Principles to Re-wire Cells*”, Zuvachem Corporation, Baltimore, Maryland, September 23, 2011.
167. Amanda Lanza and Hal Alper, “*Identifying and Characterizing High Transcription Loci in Human Cell Lines*”, Asian Congress on Biotechnology, Shanghai, China, May 14, 2011
168. Hal Alper, “*Cellular and Pathway Engineering via Directed Evolution: toward an integrative metabolic engineering approach*”, Invited Speaker, E. I. du Pont de Nemours and Company, Wilmington, DE, April 1, 2011.
169. John Blazeck and Hal Alper, “*Engineering a Fungal Conversion System for the Novel Production of Alkanes*”, American Institute of Chemical Engineers Annual meeting, Salt Lake City, UT, November 12, 2010.
170. Eric M. Young and Hal Alper, “*Improving Exogenous Sugar Utilization: a Survey of Xylose Molecular Transporters*”, American Institute of Chemical Engineers Annual meeting, Salt Lake City, UT, November 9, 2010.
171. Hal Alper, “*Using Cellular and Metabolic Engineering to Re-wire Cells for Biofuels Production*”, Invited Speaker, National Renewable Energy Laboratory, Golden, Colorado, July 14, 2010.
172. Hal Alper, “*Using Metabolic Engineering and Protein Engineering Principles to Re-wire Cells*”, Invited Speaker, Bristol-Meyers Squibb, New Brunswick, New Jersey April 2-3, 2009.
173. Hal Alper, “*Metabolic and Cellular Engineering: Rewiring cells into bio-factories*”, Invited Speaker, UT Engineering Honors Seminar, Austin, TX, February 21, 2009.
174. Hal Alper, “*Cellular and Metabolic Engineering for Biofuels and Biochemicals: Methodologies and infrastructure at UT*”, Algae to Fuels and Chemicals Workshop, UT Pickle Research Center, Austin, TX, August 15, 2008.
175. Hal Alper, “*Rewiring cells for biofuels production*”, Invited Speaker, MIT Energy Conference 2.0, Cambridge, MA, March 10, 2007.
176. Hal Alper, Gerry Fink, and Gregory Stephanopoulos, “*Engineering of cellular systems for bioethanol production using global Transcription Machinery Engineering (gTME)*”, American Institute of Chemical Engineers Annual meeting, San Francisco, CA, November 16, 2006.
177. Hal Alper and Greg Stephanopoulos, “*global Transcription Machinery Engineering: A new approach for engineering cellular systems*”, Metabolic Engineering VI, Noordwijkerhout, The Netherlands, October 5, 2006.
178. Hal Alper, Gerry Fink, and Gregory Stephanopoulos, “*Realizing cellular potential for biorefineries: Engineering tolerance and production phenotypes using global*

- Transcription Machinery Engineering (gTME)*”, The World Congress on Industrial Biotechnology and Bioprocessing. Toronto, Canada, July 13, 2006.
179. Hal Alper and Gregory Stephanopoulos, “*Defining gene knockout search trajectories: many genotypes for the same phenotype?*”, American Institute of Chemical Engineers Annual Meeting, Cincinnati, OH, November 1, 2005.
 180. Hal Alper and Gregory Stephanopoulos, “*Engineering the global regulatory networks of cells*”, American Institute of Chemical Engineers Annual Meeting, Cincinnati, OH, October 31, 2005.
 181. Hal Alper and Gregory Stephanopoulos, “*Identifying targets for pathway engineering in microorganisms*”, Invited Speaker, Gordon Research Conference on Plant Metabolic Engineering, Tilton, NH, July 15, 2005.
 182. Hal Alper and Gregory Stephanopoulos, “*Pathway optimization strategies for secondary metabolites in Escherichia coli: Lycopene bio-production as a case study*”, Invited Panel Discussion, The World Congress on Industrial Biotechnology and Bioprocessing, Orlando, FL, April 22, 2005.
 183. Hal Alper and Gregory Stephanopoulos, “*Exploring the Metabolic Landscape: Combining Systematic and Combinatorial Targets*”, American Institute of Chemical Engineers Annual Meeting, Austin, TX, November 9, 2004.
 184. Hal Alper, Kohei Miyaoku, and Greg Stephanopoulos, “*Exploring the Metabolic Landscape by Combining Rational and Combinatorial Gene Knockout Targets*”, Poster Presentation, Metabolic Engineering V, Lake Tahoe, CA, September 21, 2004.
 185. Hal Alper, Curt Fischer, Kyle Jensen, and Greg Stephanopoulos, “*Promoter Engineering: A Novel Tool for Metabolic Engineering Applications*”, Poster Presentation, Metabolic Engineering V, Lake Tahoe, CA, September 21, 2004.
 186. Hal Alper and Gregory Stephanopoulos, “*Combinatorial and Evolutionary Strategies for Pathway Optimization in Metabolic Engineering*”, American Society for Microbiology General Meeting, New Orleans, LA, May 26, 2004.
 187. Hal Alper, Yong-Su Jin, Joel F. Moxley, and Gregory Stephanopoulos, “*Identifying Targets for Metabolic Engineering*”, American Chemical Society National Meeting, Anaheim, CA, March 31, 2004.

E. Students Supervised

Current

Current Students Supervised-Graduate (13 students)

Daniel Acosta, Microbiology
Mayur Bansal, Biochemistry
Amelia Bergeson, ChE
Sarah Coleman, ChE
Natalia Estrada, Cell Molecular Biology
Angela Gordillo Sierra, ChE
Lizzie Klatt, ChE
Sung Gyung Lee, ChE
Celeste Marsan, ChE
Morgan Martinez, ChE
Ryan O'Connor, Biochemistry

Rodney Ridley, ChE
Ashli Silvera, ChE

Post-doctoral Scholars (2 Post-Docs)

Dr. Sangdo Yook
Dr. Huaimin Wang

Research Scholar (1)

Dr. Karol Nogueira

Current Students Supervised-Undergraduate (15 students)

Larissa Aspiras, UGS
Anjana Balaji, Public Health
Ryan Dittoe, ChE
Geena Kaown, Chem
Sanchali Karthikeyan, Biology
Jessica Lam, ChE
Gia Maioriello, ChE
Jenna Miller, Biology
Rebeka Nickell, Biochemistry
Blanton Priebe, ChE
Esha Ramanan, BME
Emily Riedhauser, Biology
Anupama Subramanian, ChE
Adit Trivedi, BME
Nikhil Vinod, Biochemistry

Graduated/Post-Supervision

Graduate Students Supervised (28 students)

Joseph Abatemarco, Ph.D. Chemical Engineering, 2016
John Blazeck, Ph.D. Chemical Engineering, 2013
Emily Bowman, Ph.D. Microbiology, 2023
Sierra Brooks, Ph.D., Chemical Engineering, 2023
Joseph Cheng, Ph.D. Chemical Engineering, 2016
Hannah Cole, Ph.D. Microbiology, 2023
Lauren Cordova, Ph.D. Chemical Engineering, 2020
Nathan Crook, Ph.D. Chemical Engineering, 2014
Kathleen Curran, Ph.D. Chemical Engineering, 2014
Matthew Deaner, Ph.D. Chemical Engineering, 2019
Simon D'Oelsnitz, Cell and Molecular Biology, 2021
Madeleine Flexer Harrison, M.S., Cell and Molecular Biology, 2016
Seema Irani, ChE (co-supervisor along with Yan Jessie Zhang in Biochemistry), 2019
Rebecca Knight, Ph.D., Plant Science, co-supervisor, 2014
Amanda Lanza, Ph.D. Chemical Engineering, 2013
John Leavitt, Ph.D., Cell and Molecular Biology, 2016

Sun-mi Lee, Ph.D. Chemical Engineering, 2014
Haibo Li, Ph.D., Microbiology, 2019
Leqian Liu, Ph.D. Biochemistry, 2014
Kelly Markham, Ph.D. Chemical Engineering 2018
Nicholas Morse, Ph.D. Chemical Engineering 2018
Claire Palmer, Ph.D. Cell and Molecular Biology, 2021
Kristin Presnell, Ph.D. Chemical Engineering 2023
Kevin Reed, Ph.D. Chemical Engineering, 2022
James Wagner, Ph.D. Chemical Engineering 2018
Xiunan Yi, Ph.D. Microbiology, 2023
Eric Young, Ph.D. Chemical Engineering, 2013
Shuo-Fu Yuan, Ph.D. Biochemistry, 2021

Post-doctoral Scholars (5 Post-Docs)

Aaron Lin
Hongyuan Lu
Kelly Miller
Roopa Parate
Jie Sun

Undergraduate Students (95 students)

Stephen Achilles, Biochemistry
Vaibhav Agarwala, ChE
Sabah Ali, ChE
Heming Bai, ChE
Alexandra Bailey, ChE
Hannah Baker, BME
Ramez Barsoom, ChE
Hang Bui, ChE
Rosemary Burke, BME
Jon Butler, ChE
Ishani Chakravarty, ChE
Su-Min Cho, Biochemistry
Julia Chotoo, ChE
Daniel Collins, ChE
Austin Comer, ChE
William Damkroger, ChE
Aditya Desai, ChE
Alyssa Detterman, Environmental Sciences
Jana Dsouza, ChE
Monica Duque, Biology
Tim Dyess, ChE
Ayse Erdemir, ChE
Holly Ekas, ChE
Selina Eshraghi, ChE
Elizabeth Freeman, Biology

Shruti Gadiyar, ChE
Adhitya Ganesh, ChE
Rishi Garg, ChE
Rachel Gerstner, ChE
Cedric Ginestra, ChE
Madan Gopal, ChE
Akash Gupta, ChE
Alex Hao, ChE
Delaney Hendricks, ChE
Clinton Holden, ChE
Allison Holzman, ChE
Daniel Huang, ChE
Mariam Jamoussi, ChE
Aakash Jani, ChE
Taylor Jellison, ChE
Ashty Karim, ChE
Do Soon Kim, ChE
Heath Koch, ChE
Beena Lad, Biochemistry
Lars Lauffer, ChE
Ashley Lim, ChE
Annie Liu, ChE
Sarah Ma, ChE
Chloe MacKenzie, ChE
Julio Mejia, ChE
Omar Melhem, ChE
Michael Meng, ChE
Jarrett Miller, ChE
Sayuri Monarrez Yesaki, Biochemistry
Michael Montez, ChE
Clare Murray, ChE
Priya Nair, ChE
Ankim Nguyen, ChE
Dustin Nguyen, ChE
Peter Otoupal, ChE
Annie Pan, ChE
Richard Paul, ChE
Ashley Pham, ChE
Kira Placido, ChE
Ashley Poucher, ChE
Heidi Redden, Biochemistry
Ben Reed, ChE
Samantha Refvik, Biology
Lindsey Rey, ChE
David Rives, ChE
Wenly Ruan, Deans' Scholar, Biology

Alexander Schmitz, ChE
Olivia Schmitz, ChE
Emory Siedel, ChE
Caitlin Spofford, ChE
Arvind Swaminathan, ChE
Alice Tong, ChE
Joyce Tong, Biology
Thu Tran, ChE
Cuong Tu, Biochemistry
Inem Utin, Biology
Sofia Vazquez, ChE
Maya Venkataraman, ChE
Valerie Vines, ChE
Allison Wagman, ChE
Annabel Wang, ChE
Jordan Wells, ChE
Josh Wiggers, ChE
Eden Williams, ChE
Eden Williams, ChE
Rachel Woodard, Biology
William Xu, ChE
Minye Zhao, ChE
Nijia Zhou, ChE
Andrea Zuzack, ChE

High School Students Supervised (2 student)

Divya Ramamoorthy, Westwood High School, Austin, TX
Grace Lawler, St. Michael's Catholic Academy, Austin, TX

VIII. Service

Conference Leadership Roles / Session Chairs

2024

- Member, International Metabolic Engineering Society Board Member
- Member, Society for Biological Engineering Board Member
- Chair, AIChE Division 15 Awards Committee

2023

- Conference Co-Chair, Metabolic Engineering 15 Conference
- Chair, AIChE Division 15 Awards Committee

2022

- Conference Co-Chair, Metabolic Engineering 15 Conference
- Chair, Jay Bailey Young Investigator Award Selection Committee

- Chair, AIChE Division 15 Awards Committee

2021

- Conference Chair, Metabolic Engineering 14 Conference

2020

- Conference Chair, Metabolic Engineering 13 Conference (cancelled due to COVID)

2019

- Organizing Committee, International Conference on Biomolecular Engineering

2018

- Conference Co-Chair, Metabolic Engineering 12 Conference, Munich, Germany.
- Director, AIChE Chemical Engineering Technology Operating Council (CTOC)

2017

- Program Chair, 2017 Society of Industrial Microbiology and Biotechnology Annual Meeting
- Director, AIChE Chemical Engineering Technology Operating Council (CTOC)
- Organizing Committee, 7th International Conference on Biomolecular Engineering

2016

- Division 15 Chair, American Institute for Chemical Engineers
- Lead Member, Programming Committee for Metabolic Engineering, Society of Industrial Microbiology and Biotechnology Conference
- Session Chair: “Application of ‘omics and other technologies for accelerating and enhancing bioprocess development”, ECI Cell Culture Engineering XV Conference, Palm Springs, CA, May 2016.
- Session Chair: “Session 11B: Metabolic Engineering: Methods and Applications B”, Metabolic Engineering 11 Conference, Kobe, Japan.
- Session Chair: Poster Session, Metabolic Engineering 11 Conference, Kobe, Japan.

2015

- Conference Chair, SBE’s 5th International Conference on Biological Engineering
- Division 15 Vice Chair, American Institute for Chemical Engineers
- Member, Programming Committee for Metabolic Engineering, Society of Industrial Microbiology and Biotechnology Conference

2014

- AIChE Division 15C Programming Chair
- ECI Cell Culture Engineering XIV Conference Organizing Committee Member
- Member, Programming Committee for Metabolic Engineering, Society of Industrial Microbiology and Biotechnology Conference

2013

- AIChE Division 15C Programming Vice Chair

- Member, Programming Committee for Metabolic Engineering, Society of Industrial Microbiology and Biotechnology Conference
- Chair, Poster Award Committee, ECI Biochemical Engineering XVII, Beijing, China, June 16-20, 2013
- Session Chair: “Advances in Biofuels Production: Synthetic Biology and Metabolic Engineering”, ACS National Meeting, New Orleans, LA, April 2013.
- Program Committee Member for the Metabolic Networks track of 21st Annual International Conference on Intelligent Systems for Molecular Biology, Berlin, Germany, July 2013.

2012

- AIChE Division 15C Programming 2nd Vice Chair
- Session Chair, Session 5: “Designing Cellular Circuits”, Cold Spring Harbor Asia Conference on Synthetic Biology, Suzhou, China, November 2012.
- Session Co-Chair: “15C11--Metabolic Engineering and Bioinformatics IA – Biofuels”, AIChE National Meeting, Pittsburgh, PA, October 2012.
- Session Chair: “TA004--Genomic Approaches to Systems Biology—Topical A”, AIChE National Meeting, Pittsburgh, PA, October 2012.
- Poster Session Chair, ECI Metabolic Engineering IX, Biarritz, France, June 3-7, 2012.
- Session Chair: “Advances in Biofuels Production: Synthetic Biology and Metabolic Engineering”, ACS National Meeting, San Diego, CA, March 2012.

2011

- Session Chair TA006 “Proteomics & Metabolomic Approaches to Systems Biology”. AIChE National Meeting, Minneapolis, MN, October 20, 2011.
- Session Co-Chair 15C28 “Advances In Metabolic Engineering and Bioinformatics III – Experimental”. AIChE National Meeting, Minneapolis, MN, October 17, 2011.
- Session Chair “Session 6: Biophysical and Microscale Analyses of Molecular and Cellular Function” at Society of Biological Engineering 3rd International Conference on Biomolecular Engineering, San Francisco, CA. January 19, 2011

2010

- Session Chair “15C16--Advances in Metabolic Engineering for Biofuels” session at 2010 AIChE National Meeting, Salt Lake City, UT. November 10, 2010.
- Session Co-Chair “15C20--Advances in Biocatalysis and Biosynthesis II” session at 2010 AIChE National Meeting, Salt Lake City, UT. November 10, 2010.

2009

- Session Chair, “15C16--Advances in Metabolic Engineering for Biofuels” session at AIChE meeting. Nashville, TN. November 10, 2009.
- Session Chair, “Synthetic Biology for Metabolic Engineering” session at the Society for Industrial Microbiology meeting. Toronto, Canada. July 28, 2009.
- Session Chair, “Engineering Pathways and Complex Phenotypes” session at Society of Biological Engineering 2nd International Conference on Biomolecular Engineering. Santa Barbara, CA. January 19, 2009.

Scientific Journal Reviewer

2008-2023 Reviewed journal articles for *ACS Sustainable Chemistry & Engineering*, *ACS Synthetic Biology*, *Applied Biochemistry and Biotechnology*, *Applied Microbiology and Biotechnology*, *Applied and Environmental Microbiology*, *Applied Microbiology and Biotechnology*, *Biochemical Engineering Journal*, *Biochimie*, *Biofuels*, *Biotechnology Advances*, *Biotechnology and Bioengineering*, *Biotechnology for Biofuels*, *Biotechnology Journal*, *ChemBioChem*, *Integrative Biology*, *International Research Journal of Microbiology*, *Journal of Biological Engineering*, *Journal of Biotechnology*, *Journal of Industrial Microbiology & Biotechnology*, *Journal of Theoretical Biology*, *Molecular Biology Reports*, *Molecular BioSystems*, *Molecular Biotechnology*, *Metabolic Engineering*, *Nature*, *Nature Biotechnology*, *Nature Communications*, *Nucleic Acids Research*, *PLOS Computational Biology*, *PLOS One*, *PNAS*, *Process Biochemistry*, *Science*, *Scientific Reports*, *Trends in Biotechnology*, *WIREs Systems Biology and Medicine*

Grant Review/Advisory Panels

- International Advisory Committee Member, State Key Laboratory of Bioreactor Engineering, East China University of Science and Technology, October 2018 – September 2023
- Reviewer, NSF Future Manufacturing Proposals, July 2020
- Reviewer, AAAS Research Competitiveness Program, January 2018
- Reviewer, Israeli Science Foundation, February 2017
- Reviewer, DOE JGI Synthesis Grants, September 2016
- Reviewer, DOE-Basic Engineering Science Grants, March 2016
- Ad Hoc Reviewer, NSF CBET Division, February 2016.
- Reviewer, NIH IMST 15: Cell, Molecular, and Computational Biology Small Business study section, June 2015
- Reviewer, DOE TABB Program, March 2015
- Reviewer, DOE SBIR Program, August 2012
- Reviewer, DoD Strategic Environmental Research and Development Program (SERDP), April 2012
- Reviewer, Israel Science Foundation, March 2012
- Reviewer, BP Energy Biosciences Institute, August 2011.
- Reviewer, DOE ARPA-E program, June 2011
- NSF Review Panelist, CBET Division, May 2011
- DOE Early Career Award Review Panelist, February 2011
- Ad hoc reviewer, USDA SBIR program, February 2011
- Reviewer-2010-2011 UT-Austin Research Grants
- Ad hoc reviewer, NSF, Molecular Biochemistry Division, September 2010
- NSF Review Panelist, CBET Division, June 2010
- NSF Review Panelist, CBET Division, January 2010

Thesis Committees

- Kevin Baldrige (ChE, Contreras)
- Sean Carroll (ChE, Georgiou)
- Xi Chen (ChE, Rochelle)
- Sung Hee Cho (CMB, Contreras)
- Constantine Chrysostomou (ChE, Georgiou)
- Chase Cornelison (ChE, Schmidt)

- Blair Cox (ChE, Ekerdt)
- Shira Cramer (ChE, Georgiou)
- Christopher Dundas (ChE, Keitz)
- Alyssa Ekdahl (ChE, Contreras)
- Peter Enyeart (CMB, Ellington)
- Zach Frye (ChE, Maynard)
- Mark Gebhard (ChE, Georgiou)
- Grant Gelderman (ChE, Contreras)
- Jon Gengler (Biochemistry, Liu)
- William Kelton (ChE, Georgiou)
- Daniel Klein-Marcuschamer (MIT ChE, Stephanopoulos)
- Rebecca Knight (Plant Biology, Brand)
- Byoung Joon Ko (ChE Mullins/Brodbelt)
- Abigail Leistra (ChE, Contreras)
- William Liechty (ChE, Peppas)
- Jiwon Lee (ChE, Georgiou)
- Wei-Cheng Lu (CMB, Ellington)
- Andre Maranhao (CMB, Ellington)
- Adam Meyer (CMB, Ellington)
- Justine Murray (Microbio, Whiteley)
- Jennifer Pai (ChE, Maynard)
- Olga Paley (ChE, Georgiou)
- Gregory Palmer (CMB, Whitley)
- Ahlam Qerqez (ChE, Maynard)
- Erik Quandt (CMB, Georgiou)
- Aaron Rozelle (ChE, Lee)
- Brian Renda (CMB, Ellington)
- Jamie Sutherland (ChE, Maynard)
- Drew Tack (Biochemistry, Ellington)
- Rayna Taniguchi (BME, Fenno/Santacruz)
- Bailey Tibbett (ChE, Keitz)
- Richelle Thomas (ChE, Schmidt)
- Chen-Hsun Tsai (ChE, Contreras)
- Jorge Vazquez (ChE, Contreras)
- Drew Wagner (Biochemistry, Keatinge-Clay)
- Bo Wang (ChE, Georgiou)

Departmental and University Committees

- Chair of Search Committee, Graduate Dean and Senior Vice Provost, 2023
- Graduate Studies Committee Executive Committee Member, 2022-2025
- Chair of Search Committee, Associate Vice President for Research-Innovation & Economic Development, 2020
- Secretary, Graduate Assembly, 2020-2022
- Graduate Assembly Representative, 2019-2022
- Executive Committee Member, Faculty Council, 2017-2018
- Chair, Graduate Assembly, 2017-2018 term

- Chair, Graduate Assembly Academic Committee, 2016-2017
- Member, ICMB and Biomedical Research Core Facilities Director Search Committee, 2016
- #1 Committee, McKetta Department of Chemical Engineering, 2015-2016
- Cell and Molecular Biology Qualifying Examination Revamp Committee, 2015
- Cockrell School of Engineering Medical School Advisory Group, 2013-2014
- Graduate Assembly Academic Committee Member, 2015-2018
- Graduate Assembly Admissions Committee Member, 2012-2015
- Graduate Assembly Representative, 2012-2018
- Chemical Engineering Graduate Student Recruiting and Admissions Committee, 2011-2012
- Interdisciplinary Systems Biology Hiring Committee, 2011-2012
- Kinetics Qualifying Exam Committee (Fall 2011-current)
- Member, Drug Discovery and Manufacturing Initiative Exploratory Committee (2011)
- Member and Chair, EERC Interdisciplinary Teaching Space Design Committee (2010-2011)
- Chemical Engineering Faculty Search Committee, 2010-2011
- External Member, Biomedical Engineering Department Faculty Search Committee, 2009-2010
- Cell and Molecular Biology Graduate Admissions Committee (2010-current)
- ChE Area Option Curriculum Revision Committee, Spring 2009
- Transport Qualifying Exam Committee (Fall 2008-Spring 2011)
- Focus Group #5: Biofuels created by Dean Fenves for Dr. Robert Orbach, March 2009
- Core Member, Cockrell School of Engineering Focus AREA 2: Engineering Human Health and Health Care Delivery, Spring 2009
- Member, ChE Outcome Evaluation Committee 1 for ABET Criteria Monitoring

Additional University Service

- Platform Party Member, Spring 2018 University Commencement Ceremony
- Graduate Marshall, Spring 2016 Engineering Commencement Ceremony

Additional Teaching Service

- Faculty Coordinator, ChE185, Graduate Student Seminar Series, 2010-2017
 - Raised \$7000 for 2010-2011 seminar series from corporate sponsors
 - Raised \$8500 for 2011-2012 seminar series from corporate sponsors
 - Raised \$7500 for 2012-2013 seminar series from corporate sponsors
 - Raised \$5250 for 2013-2014 seminar series from corporate sponsors
 - Raised \$3250 for 2014-2015 seminar series from corporate sponsors
 - Raised \$1750 for 2015-2016 seminar series from corporate sponsors
 - Raised \$3250 for 2016-2017 seminar series from corporate sponsors
 - Raised \$8250 for 2016-2017 seminar series from corporate sponsors
- Guest Presentation, ChE102, Introduction to Chemical Engineering, “Cellular and Metabolic Engineering: From Biofuels to BioPharmaceuticals”, Each Fall from 2009 - 2014
- Guest Lecture, in Biology of Biofuels Freshmen Research Initiative, “Cellular and Metabolic Engineering: From Biofuels to BioPharmaceuticals”, April 11, 2011
- Facilitated Graduate School Information Session for OXE Chemical Engineering Honor Society, September 30, 2010, October 4, 2011, and October 8, 2012.
- Guest Lecture in ChE339, Biochemical Engineering, “Biofuels: Overview, Opportunities and the Future”, November 12, 2009

- Faculty Panel, Rockin' Pipettes, a WEP Chemical Engineering FIG (4/22/09) and The Processors, a Chemical Engineering FIG (10/13/09)
- Guest Lecture, in CH391L, Metabolism 2009, “Metabolic Engineering: Major advances and ongoing research”, October 15, 2009
- Guest lecture for ChE339, Biochemical Engineering, “Biofuels: Overview, Opportunities and the Future”, November 25, 2008

Student Organization Advising

- Faculty Advisor, Society for Biological Engineers Student Chapter

ANNEX B

Prior Expert Witness work that has led to depositions and/or trial

DSM IP ASSETS, B.V. & DSM BIO-BASED PRODUCTS & SERVICES B.V., Plaintiffs,
vs.
LALLEMAND SPECIALTIES, INC. & MASCOMA LLC, Defendants
Civil Action No. 3:16-cv-497
May 2018

Expert witness for DSM

ZHEJIANG MEDICINE CO. LTD., and ZMC-USA L.L.C., Plaintiffs,
vs.
KANEKA CORPORATION, Defendants
Civil Action No. 4:11-CV-1052
January 2015

Expert witness for Validity for Kaneka

Ongoing Work:

Currently Retained as a Witness for:
IMPOSSIBLE FOODS INC., Plaintiff,
vs.
MOTIF FOODWORKS, INC., Defendant
Civil Action No. 22-cv-311

Expert witness for Impossible Foods Inc.