Title
Engineering a Yeast-Based Platform for Production of Novel Monoterpene Indole Alkaloid Analogs

Author
Misa, Joshua Russell

Publication Date
2023

Peer reviewed|Thesis/dissertation

UNIVERSITY OF CALIFORNIA

Los Angeles

Engineering a Yeast-Based Platform for Production of Novel Monoterpene Indole Alkaloid Analogs

A dissertation submitted in partial satisfaction of the requirements for the degree Doctor of Philosophy in Chemical Engineering

by

Joshua Russell Misa

2023

ABSTRACT OF THE DISSERTATION

Engineering a Yeast-Based Platform for Production of
Novel Monoterpene Indole Alkaloid Analogs

by

Joshua Russell Misa

Doctor of Philosophy in Chemical Engineering

University of California, Los Angeles, 2023

Professor Yi Tang, Chair

In addition to satisfying nutritional needs, humans have been consuming plants for medicinal and recreational purposes for millennia. The medicinal and recreational properties of plants are attributed to compounds that are not a product of the plant's core metabolism, but are rather secondary metabolites, also known as natural products. Monoterpene indole alkaloids (MIAs) are an expansive class of bioactive plant natural products, many of which have been named on the World Health Organization's List of Essential Medicines. Among MIAs' divergent structural complexity are psychoactive MIAs such as ibogaine and mitragynine which also hold therapeutic potential. However, low production from native plant hosts necessitates a more reliable source of these compounds to meet global demands in medicine and research. The recent explosion of synthetic biology toolsets and genomics data has enabled reconstitution of plant biosynthetic pathways to build complex MIA structures in alternative hosts.

In this dissertation, we report on the development of a yeast-based platform for high-titer production of the universal MIA precursor, strictosidine. Our fed-batch platform produces ~50 mg/L strictosidine, starting from the commodity chemicals geraniol and tryptamine, and is the highest titer reported to date. Next, we describe approaches to further optimize this platform and leverage it to produce strictosidine analogs. Bioprospecting homologs of pathway genes reveal the variants from *Catharanthus roseus* have the highest activity in yeast. Finally, we utilized our strictosidine platform to access bioactive MIAs such as heteroyohimbine and corynantheidine-type MIAs. We also demonstrate our ability to access novel analogs of these compounds with our platform, which potentially have improved or divergent bioactivity from their native forms.

The dissertation of Joshua Russell Misa is approved.

Neil K. Garg

Junyoung O. Park

Todd O. Yeates

Yi Tang, Committee Chair

University of California, Los Angeles

2023

DEDICATION

To Leroy and Carole Russell,

Thank you for all your love and support,

I love you.

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

ACKNOWLEDGEMENTS

Section 1 contains material written by Misa, J. from the following publications:

Jamieson, C. S., Misa, J., Tang, Y. & Billingsley, J. M. Biosynthesis and synthetic biology of psychoactive natural products. Chem. Soc. Rev. 50, 6950–7008 (2021).

Misa, J., Billingsley, J. M., Niwa, K., Yu, R. K. & Tang, Y. Engineered Production of Strictosidine and Analogues in Yeast. *ACS Synth. Biol.* **11**, 1639–1649 (2022).

Section 2 and Section 3 contain material written by Misa, J. from the following publication:

Misa, J., Billingsley, J. M., Niwa, K., Yu, R. K. & Tang, Y. Engineered Production of Strictosidine and Analogues in Yeast. *ACS Synth. Biol.* **11**, 1639–1649 (2022).

The work described in this dissertation was supported by the National Institute of Health (NIH) grant R01AT010001-0, as well as the NIH National Institute of General Medical Sciences predoctoral fellowship T32 GM136614.

The past five years have been the most difficult but rewarding journey of my life so far. Traversing through this rollercoaster of emotions and mental states is something I could not have done alone. The proverb “it takes a village to raise a child” comes to mind, and I believe it applies to a PhD as well. There are countless people whose direct and indirect support have enabled me to get to this final stage and whom I am eternally grateful for.

Firstly, I would like to extend deep gratitude to my advisor Professor Yi Tang. His mentorship and guidance in both research and professional development has greatly shaped me into the scientist I am today. My first impression of Prof. Tang was from one of his former students,

Yanran Li, an associate professor at my alma mater UCR back in 2018. I reached out to her to get some insight into Prof. Tang's mentorship style and get an idea of what to expect when working in the Tang group. She only had warm and glowing words to share about Prof. Tang and her time as a PhD student, even when describing her early struggles in the program. The support and guidance in the face of adversity Prof. Tang offered her in those critical moments was powerful and something that stuck with me. After five years working with Prof. Tang, it is something I can affirm first-hand as well. Prof. Tang has the distinct ability to adapt his mentorship style to each individual lab member. From day one I felt he understood and trusted my independence as a researcher which provided a space for me to explore science unencumbered. Early in my graduate school journey, like many students, I was paralyzed by imposter syndrome. Prof. Tang assured me that I was just as competent of a scientist as everyone else in the lab and emphasized to me to not make unfair comparisons to more senior members in the lab. Lastly, the most valuable skill Prof. Tang has instilled in me is the art of science storytelling, seeing the data and results for more than just numbers but rather plot points in a logical progression of information. This skill helped secure my passion and appreciation for science and the wonderful stories to uncover. Thank you, Prof. Tang, for the opportunity to grow into a better scientist.

Next, I would like to provide sincere thanks to my PhD committee: Prof. Garg, Prof. Park, and Prof. Yeates for their support, encouragement, and scientific insight with various fellowship applications and throughout my PhD.

I am extremely thankful for Team Stricto and all their comradery and mentorship. You are all the most talented team of scientists and it's been a privilege to work with each of you. John Billingsley, thank you for your mentorship and guidance not only in my first year when you were a student, but throughout the years in your capacity as a visiting scientist. I always appreciate you checking in on my project progress and giving me new direction when I feel like I hit a wall. Undramaa Bat-Erdene, thank you for your support and teaching me basics of protein expression.

Danielle Yee, the “Yeast Whisperer,” thank you for providing endless tips and tricks for working with yeast and helping shape me into the yeast engineer I am today. I learned so much from you and I am grateful to have gotten to work with you for most of my PhD. Moriel Dror, thank you for all your hard work and accepting the mantle of the project. You are one of the most resilient and determined researchers I’ve met, and I know you are going to take the project to new heights.

I would like to next thank my cohort mates Dmitriy Ruckodanov and Ikechukwu Okorafor. I will never forget all the great memories at trivia nights throughout the years. We’ve gone by many names, but “Josh and the Jersey Boys” is a near and dear favorite. Our string of victories really had us eating well. Dima, you were my first new friend when coming to UCLA and I will always appreciate the memories together at GME, late night Halo playthroughs, and board game nights. It was also a privilege to assist you in your own dissertation project and teach you the ways of microbial biology. Ike, my fellow Class of 2023, thank you for all the support and friendship throughout the years. I will cherish the great conversations and discussions about basketball with Nick and I. Late night chats in 7564 will always be a fond memory.

I wanted to acknowledge all my mentees, who (as cliché as it is) have taught me just as much as I’ve taught you. Michael Guile and Christine Minor, you both taught me how to be a better mentor and find new ways of teaching. I greatly appreciated working with both of you in the short time we overlapped. Moriel, you put up with me and my teaching during a time when I felt I wasn’t at my best. You are such a quick learner and seeing you rise into an independent researcher has been so fulfilling to watch. Lastly, Rachel Yu, thank you for your pivotal support inside and outside of the lab. Your contributions to the strictosidine project, both in assisting my experiments and leading an independent route of your own, have been essential to Team Stricto’s success. You are an exceptional researcher and scientist. Your genuine curiosity for science and appetite for answers is infectious and helped reignite my own passion for science during the time I lost mine.

I am lucky to also call you one of my closest friends and our friendship over the past three years has been vital towards the success of my PhD journey. I am eternally grateful for your support.

Next, I would like to extend my gratitude to the rest of the Tang lab members I've had the distinct privilege to work with throughout my five years. Masao Ohashi, thank you for all the great conversations about science and life, many of which took place way too late at night. You are the most talented scientist I know, and I've learned so much from your approach and analytical process. Every researcher you come in contact with is better because of it, just by pure osmosis. Masa, you have some of the best dry humor I've ever heard, with impeccable timing, thank you for all the laughs. Nicholas Liu, my desk neighbor for the first half of my PhD journey, thank you for all the great discussions about basketball, life, and (sometimes) science. Everyone else in the Tang lab, you have all impacted or taught me something in some way. Thank you all for making my time in the Tang lab so enjoyable and full of great memories.

I must also thank my close friends, all of whom I've met throughout different periods of my life but will always be connected with. Bret Gallwey, Daniel Yambot, Doni Tadesse, Surya Kumaraguru, Dmitriy Ruckodanov, Rachel Yu, Ashley Sun, and Michelle Hsieh, thank you from the bottom of my heart for all the memories and for helping me find escape in your own ways. I am fortunate to be able to connect with each of you through various hobbies or passions. You are all a core part of my support structure, and I couldn't be where I am without you. I sincerely appreciate all of you for your patience with all the canceled plans, rain checks, and turned down hangouts in sacrifice for my studies, but now there will be plenty of time to make more great memories together. You all mean the world to me, and I love you all so much.

Finally, I would like to thank my family. I love you all more than I could ever put into words, and I am so lucky to have you all in my life. Thank you, Mom and Dad, for providing for me and allowing me to focus solely on my education for the past 20 years. My studies being my primary

focus is a privilege not many students have, especially in higher education, so I will always be grateful for that. Thank you, Nana, Sarah, and Sammy, my amazing sisters who have provided me with a lifetime of happy memories and distractions from the real world through quality time together. There's nothing like relieving stress from graduate school like stressing each other out with Overcooked, Tetris, or Mario Kart games.

VITA

2014-2018	University of California, Riverside B.S. in Chemical Engineering with emphasis in Biochemical Engineering Chancellor's Scholar, *magna cum laude* Riverside, CA
2017-2018	Chancellor Research Fellowship University of California, Riverside Riverside, CA
2019	NSF Graduate Researcher Fellowship Program – Honorable Mention
2020-2022	NIH Chemistry-Biology Interface Predoctoral Training Fellowship University of California, Los Angeles Los Angeles, CA
2020-2022	NSF BioPacific MIP Fellowship University of California, Los Angeles Los Angeles, CA
2022-2023	Dissertation Year Fellowship University of California, Los Angeles Los Angeles, CA

PUBLICATIONS

Schwartz, C., Frogue, K., Ramesh, A., Misa, J. & Wheeldon, I. CRISPRi repression of nonhomologous end-joining for enhanced genome engineering via homologous recombination in *Yarrowia lipolytica*. *Biotechnol. Bioeng.* **114**, 2896–2906 (2017).

Schwartz, C., Frogue, K., Misa, J. & Wheeldon, I. Host and pathway engineering for enhanced lycopene biosynthesis in Yarrowia lipolytica. *Front. Microbiol.* **8**, (2017).

Misa, J., Schwartz, C. & Wheeldon, I. Design of Hybrid RNA Polymerase III Promoters for Efficient CRISPR-Cas9 Function. *Bio-Protocol* **8**, 1–12 (2018).

Misa, J. & Schwartz, C. CRISPR Interference and Activation to Modulate Transcription in Yarrowia lipolytica. in *Yarrowia lipolytica: Methods and Protocols* 95–109 (2021).

Jamieson, C. S., Misa, J., Tang, Y. & Billingsley, J. M. Biosynthesis and synthetic biology of psychoactive natural products. Chem. Soc. Rev. 50, 6950–7008 (2021).

Misa, J., Billingsley, J. M., Niwa, K., Yu, R. K. & Tang, Y. Engineered Production of Strictosidine and Analogues in Yeast. *ACS Synth. Biol.* **11**, 1639–1649 (2022).

PRESENTATIONS

Misa, J., & Tang, Y. Engineering a yeast-based platform for production of novel monoterpene indole alkaloid analogs. 2020 Molecular Biology Institute Retreat, Virtual, Poster Session. *Poster Award Recipient*.

Misa, J., & Tang, Y. Engineering a yeast-based platform for production of novel monoterpene indole alkaloid analogs. 2021 Sigman Symposium, Virtual, Poster Session.

Misa, J., & Tang, Y. Engineering a yeast-based platform for production of novel monoterpene indole alkaloid analogs. 2021 Molecular Biology Institute Retreat, Virtual, Poster Session.

1. INTRODUCTION

The use of microbial factories to produce high-value pharmaceuticals derived from plant natural products is enabled by recent advancements in synthetic biology and metabolic engineering Baker's yeast, *Saccharomyces cerevisiae*, has proven to be a particularly powerful industrial host due to its generally regarded as safe (GRAS) status, genetic tractability, and scalability.[1] Yeast is also an attractive host because it shares a similar endomembrane system with plants, which allows for heterologous expression of plant cytochrome P450 enzymes that are often responsible for generating the chemical complexity that confers potent biological activity.[2,3] Recently, a number of complex plant natural products have been produced from engineered yeast, including tropane alkaloids such as scopolamine,[4] benzylisoquinolines such as hydrocodone[5] and noscapine,[6] sesquiterpene lactones such as artemisinin,[7] and monoterpene indole alkaloids (MIAs).[8–10]

Strictosidine is the universal precursor to thousands of structurally diverse MIAs found across many plant families (Figure 1).[11] A notable MIA producer is the flowering subshrub, *Catharanthus roseus,* from the *Apocynaceae* family, which is known to biosynthesize the potent anti-cancer natural products vincristine and vinblastine.[11] However, these bioactive MIAs, as well as strictosidine itself, accumulate at trace amounts in their native producers and are difficult to isolate. Given its central role in the biosynthesis of MIAs, access to a scalable route for producing strictosidine is highly desirable for both research and industrial applications. While a number of strategies have been developed to chemically synthesize strictosidine and analogs,[12–14] these multistep routes are difficult to scale and have low overall yields. Yeast expressing strictosidine synthase (STR) was used in the biotransformations of secologanin from plant extracts into strictosidine.[15,16] However, secologanin is prohibitively expensive as a pure starting material, while the plant extracts are not readily available or scalable. Hence, microbial biosynthesis of strictosidine from easily accessible starting materials is an attractive approach.

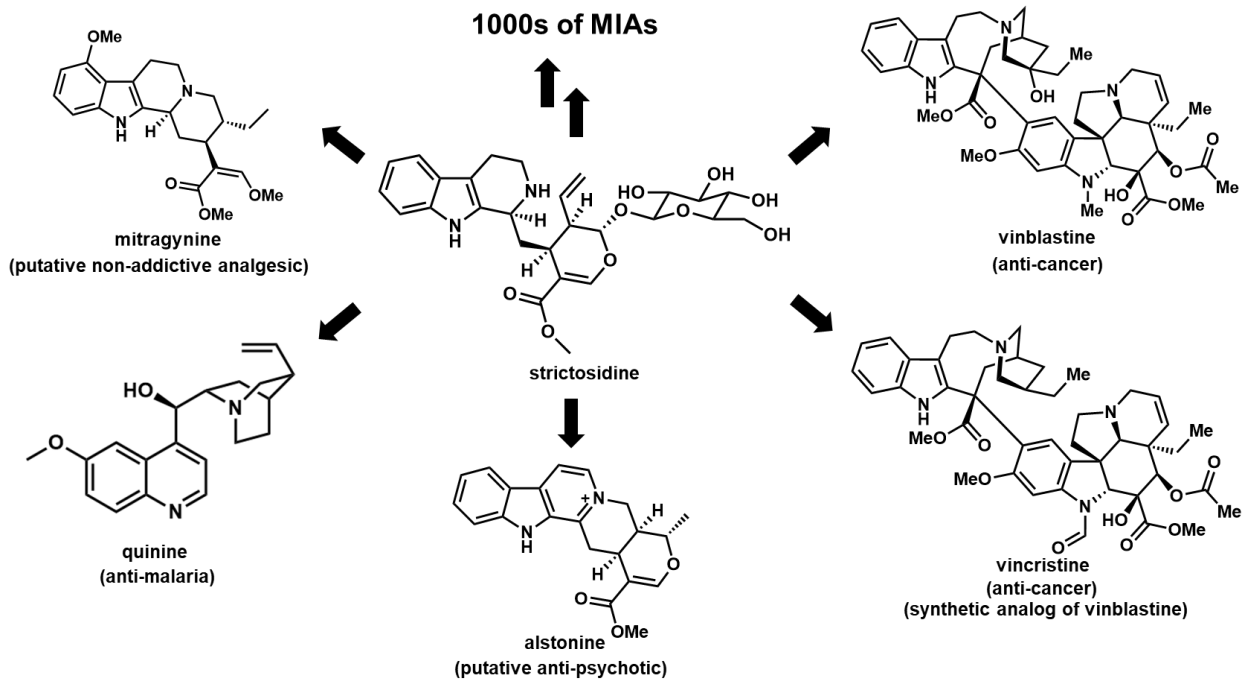


Figure 1. Strictosidine is the universal MIA precursor.

1.1. Biosynthesis of Monoterpene Indole Alkaloids

Given that strictosidine is the central metabolite in the MIA biosynthetic pathways in plants, there has been intense efforts to understand how nature transforms the simple geranyl (C10) precursor that combines with tryptamine to yield the complex strictosidine. These efforts from different labs have fully elucidated the strictosidine pathway. In recent years, further efforts have led to the complete mapping of the downstream enzymatic transformation to vinblastine in C. roseus, comprised of over 30 enzymes starting from primary metabolites.[17–25] Shortly after, the complex (−)-ibogaine biosynthetic pathway was also elucidated, as well as other structurally diverse psychoactive MIA compounds such as kratom alkaloids from *Mitragyna speciosa*.[26,27]

The first committed step in the seco-iridoid pathway towards the monoterpene scaffold in strictosidine is the formation of geraniol (Figure 2.). While it was predicted that geraniol was hydrolyzed from the mevalonate pathway intermediate, geranyl pyrophosphate (GPP)[28,29] the enzymatic basis of its formation was unknown until the discovery of geraniol synthase (GES) from sweet basil (*Ocimum basilicum*) decades later.[30] Since then, many GES homologs have been

discovered in various plants. The activity of GES, which is to hydrolyze GPP to geraniol, represents a divergence point between primary and secondary terpene metabolism in plants. In primary metabolism, GPP is further elongated to farnesyl pyrophosphate (FPP), which is central to the synthesis of steroids and coenzyme Q. By hydrolyzing the pyrophosphate in GPP, GES commits the geraniol group for MIA biosynthesis and siphons GPP away from primary metabolism. In the MIA pathway, geraniol is then hydroxylated by the P450 enzyme geraniol 8-hydroxylase (G8H) to form 8-hydroxygeraniol.[31]

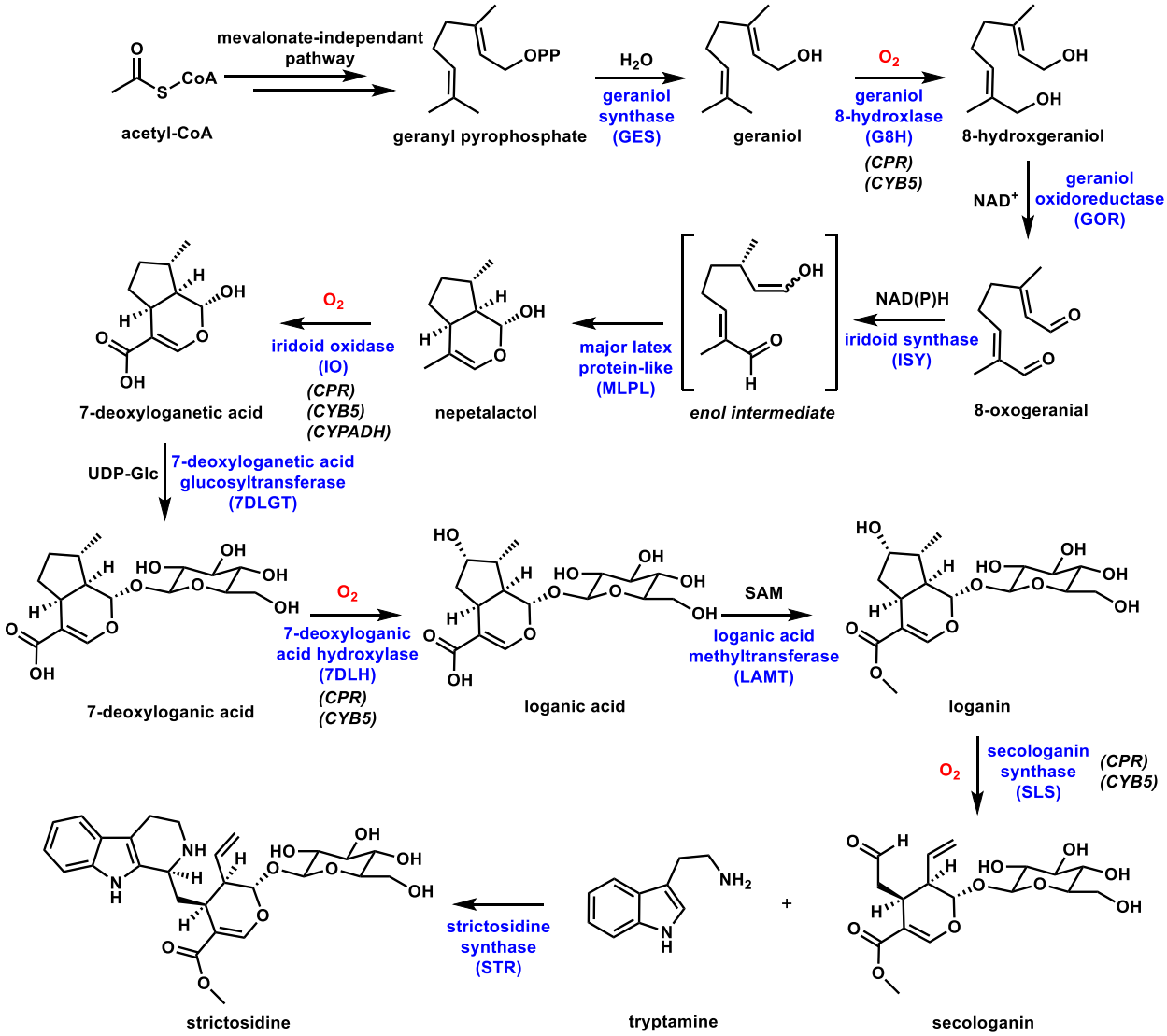


Figure 2. Biosynthetic pathway of strictosidine from primary metabolism.

The next four biosynthetic steps were all discovered from analysis of the C. roseus transcriptome.[17] 8-Hydroxygeraniol oxidoreductase (GOR) iteratively oxidizes the two alcohols in 8-hydroxgeraniol to yield 8-oxogeranial, a dialdehyde that is poised for intramolecular cyclization. It was initially believed that iridoid synthase (ISY) was a NAD(P)H-dependent cyclase.[32] However, a recent report demonstrated that ISY is a reductase that can reduce 8-oxogeranial to an enol intermediate.[33] A previously undiscovered cyclase, major latex protein-like (MLPL), then facilitates the cyclization of the reduced enol to form cis–trans nepetalactol via a non-cofactor dependent mechanism.[25] Nepetalactol is the first molecule in the pathway that has the iridoid structure. In plants such as *Nepeta*, nepetalactol can be oxidized to neptalactone, which is the cat attractant produced by these plants.[33] In the MIA pathway nepetalactol undergoes a 4-electron oxidation catalyzed by the P450 iridoid oxidase (IO) to install an α,β-unsaturated carboxylic acid in 7-deoxyloganetic acid. The next step is glucosylation by 7-deoxyloganetic acid glucosyl transferase (7DLGT) with uridine diphosphate-glucose (UDP-glucose) to form 7-deoxyloganic acid. Glucosylation of the hemiacetal presumably stabilizes the compound and prevents spontaneous ring opening. The third P450 in the pathway, 7-deoxyloganic acid hydroxylase (7DLH), catalyzes hydroxylation of the cyclopentane ring in 7-deoxyloganic acid to form loganic acid.

Expression data revealed that the next two genes in the seco-iridoid pathway encoding for loganic acid, O-methyltransferase (LAMT) and secologanin synthase (SLS), are part of a separate regulon from the early pathway.[34,35] The seco-iridoid pathway is also spatially segmented between the internal phloem associated parenchyma (IPAP) cells for iridoid production and leaf epidermis cells for the remaining steps towards production of strictosidine.[36] Loganic acid is first transported from the cytosol of the IPAP cells into the cytosol of epidermic cells by a nitrate/peptide family (NPF) transporter.[37] The cytosolic LAMT subsequently converts loganic acid into loganin.[34] The fourth P450 in the pathway, SLS then catalyzes oxidative cleavage of the cyclopentanol ring of loganin to unveil the reactive aldehyde handle in secologanin.[38]

To form strictosidine, secologanin and tryptamine are condensed through a stereospecific Pictet–Spengler reaction catalyzed by strictosidine synthase (STR).[39] This mechanism had been long proposed before the discovery of STR, modeled after the formation of L-benzylisoquinolines alkaloids.[40] Considering the synthetic challenges associated with accessing strictosidine, STR has become an attractive enzyme for the chemoenzymatic and biotransformative syntheses of analogs.[41–44] The regulation and complexity of MIA biosynthesis is further highlighted by the transient sub-cellular compartmentalization of strictosidine formation in the vacuole of epidermis cells followed by immediate export towards the nucleus.[45]

It is within the nucleus that the next enzyme catalyzed transformation of the MIA scaffold takes place, the removal of the glucose moiety from strictosidine to form strictosidine aglycone by strictosidine-O-β-glucosidase (SGD) (Figure 3).[46] It is believed that the spatial isolation of STR and its substrates from SGD prevents accumulation of the highly-reactive strictosidine aglycone intermediate, 4,21-dehydrogeissoschzine, a dialdehyde which leads to toxic protein cross-linking.[47] It is hypothesized that this is a plant defense mechanism from herbivores mirroring the activation of the related phenolic secoiridoid glycoside, oleuropein, from the privet tree, *Ligustrum obstusifolium,* following tissue damage.[48]

Whereas strictosidine is relatively stable and benign to the host, removal of the glucose group which essentially serves to mask the hemiacetal, leads to one of the strictosidine aglycone forms, the dialdehyde 4,21-dehydrogeissoschizine that is prone to protein cross-linking. It exists in equilibrium with the more stable epimers cathenamine and strictosidine aglycone (open form).[49] Each of these aglycone intermediates represents a divergence point towards different terminal alkaloids.[23,50] For example, 4,21-dehydrogeissoschizine is the strictosidine aglycone form towards iboga alkaloids. This class of MIAs can then branch towards the potent anti-cancer drug vinblastine, or towards the psychoactive compound ibogaine.

Another class of MIAs from strictosidine aglycone intermediates are the heteroyohimbine alkaloids. First characterized from the flower *Rauvolfia serpentina,* MIAs of this class have a wide

variety of bioactivity. Preparations of this plant have been used in India for centuries to treat hypertension, malaria, snake bites and more.[51] In the past 50 years, investigations into the alkaloid content have revealed MIAs responsible for some of the above bioactivities. Biosynthesis begins from one of the lactone ring-closed strictosidine aglycone forms, cathenamine, which then undergoes a reduction catalyzed by tetrahydroalstonine synthase (THAS) or heteroyohimbine synthase (HYS) to yield tetrahydroalstonine and the anti-hypertensive drug, ajmalicine, respectively (Figure 3).[52] Tetrahydroalstonine can then be oxidized by alstonine synthase (AS) to form the anti-psychotic compound, alstonine. Following oxidation of the tryptamine-derived backbone to form what is known as the β-carboline scaffold, alstonine is fluorescent, which could potentially enable it to be used as a molecular probe for microbial MIA production *in vivo*. Combined with other synthetic biology tools, such a probe can be leveraged for high-throughput engineering approaches such as enzyme evolution.

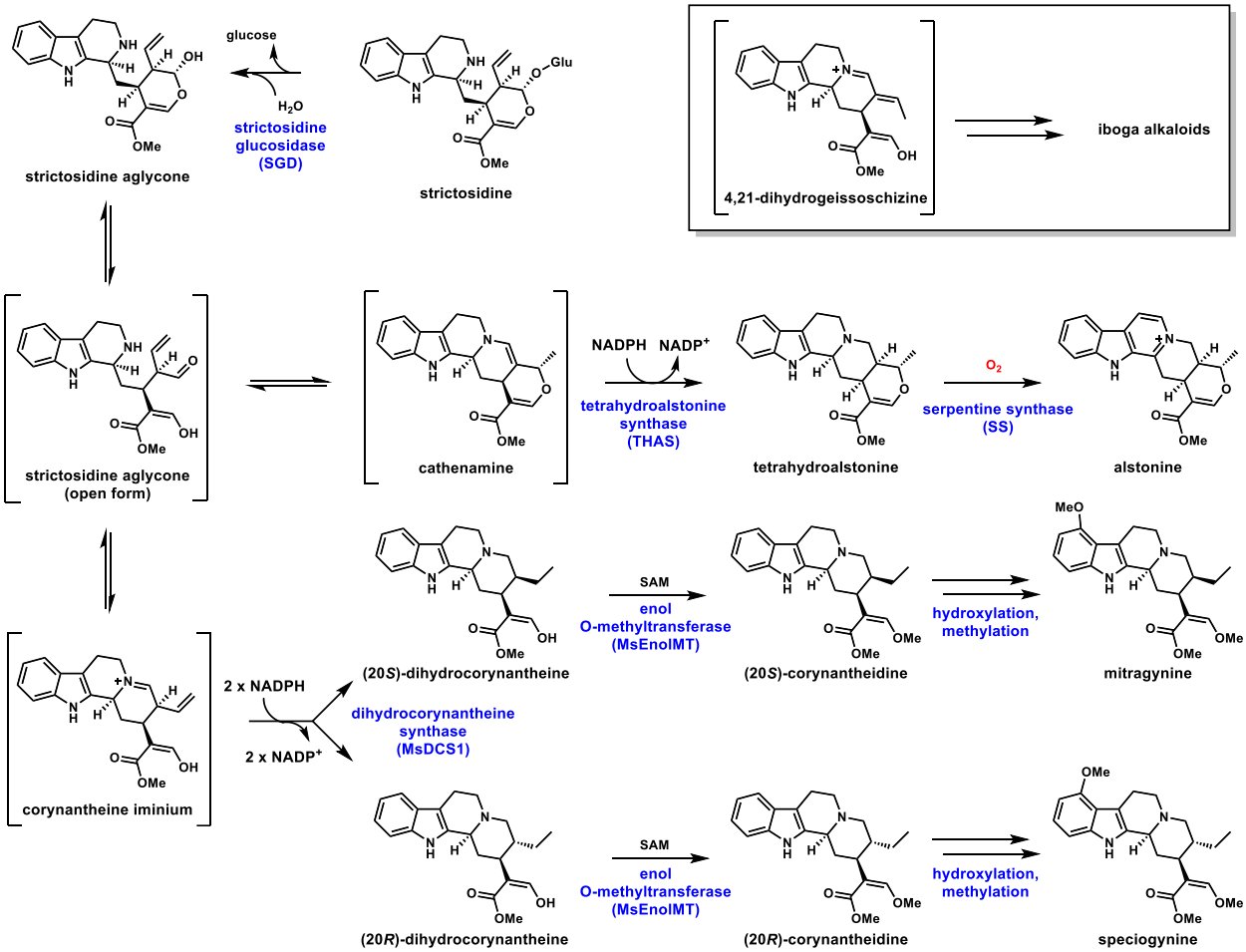


Figure 3. Divergent biosynthetic pathways starting from strictosidine aglycone.

Another set of MIAs of increasing interest are those from the tropical evergreen tree, *Mitragyna speciosa*, colloquially known as kratom. More than 50 corynanthe-type MIAs (also referred to as kratom alkaloids) have been isolated from the *Mitragyna speciosa* plant, several of which exhibit opioid-like properties.[53] Native to Southeast Asia, kratom has been used in traditional Thai medicine for centuries. The use in the United States has increased rapidly since early 2000s, both recreationally and to relieve chronic pain or opioid withdrawal symptoms. Compared to conventional opium alkaloids, kratom alkaloids exhibit "unique binding and functional profiles" suggesting that plant extracts may be effective alternatives to the benzylisoquinoline-based (opioid) pain treatments.[54] However, similar to opium alkaloids, repeated use of kratom may lead to addiction, and the FDA has not approved kratom for any

medical use; as a result, the DEA lists kratom as a Drug of Concern. The first reported and most abundant kratom alkaloid is mitragynine, comprising up to 66% of the alkaloid content in Thai cultivars.[55] Kratom alkaloid biosynthesis starts with the 1,2 and 1,4-reductions of the corynantheine iminium form of strictosidine aglycone catalyzed by medium-chain alcohol dehydrogenases known as dihydrocorynantheine synthases (DCSs) (Figure 3).[27] There are three known orthologs of DCS, one from *Cinchona pubescens* (CpDCS) as part of the quinine biosynthetic pathway, and two from *M. speciosa* (MsDCS1 and MsDCS2), with varying stereo outcomes. CpDCS and MsDCS2 both show near total formation of the (20*R*)-dihydrocorynantheine, while MsDCS1 interestingly shows formation of both (20*S*) and (20*R*) isomers albeit with large variation between experiments.[27] Next in the pathway, methylation of dihydrocorynantheine isomers by an enol-O-methyltransferase (MsEnolMT) results in the formation of the respective corynantheidine isomer. To access the most potent kratom alkaloids, stereoisomers speciogynine and mitragynine, a methoxy group must be installed onto the 4-position of the indole ring on the (20*R*) and (20*S*)-corynantheidine scaffolds, respectively. It is predicted that first, a P450 hydroxylation of the 4-position, followed by methylation catalyzed by an O-methyltransferase would afford speciogynine and mitragynine. However, those enzymes have yet to be elucidated.[27]

1.2. Yeast as a Microbial Factory

A critical parameter in the successful refactoring of a natural product pathway is the selection of a suitable biosynthetic chassis. There are several considerations that need to be made ranging from robustness, biosynthetic pathway compatibility, genetic tractability, and more. However, one must carefully consider the features of a given pathway before deciding if a particular chassis meets the biosynthetic requirements. One chassis, *Escherichia coli*, the model bacterium has become a foundation of biotechnology as a DNA bearing model organism. *E. coli* strains are commonly customized for plasmid propagation and protein expression, but using *E.*

coli for the production of drugs with relatively short biosynthetic pathways have been shown with stepwise mixed-strain cultures leveraged for longer pathways.[56–58] One attribute that attenuates *E. coli* as a chassis for more complex biosynthetic pathways is the lack of an endomembrane network found in eukaryotic cells that allows for expression of transmembrane enzymes. Cytochrome P450s are the largest family of transmembrane proteins and are pervasive in primary and secondary metabolism. While efforts have been made to express some truncated and evolved variants of P450s in a soluble state in E. coli, a eukaryotic host is superior in this regard.[59]

Saccharomyces cerevisiae (Baker's yeast) has become a favorite organism among academics and industry professionals alike for its ability to demonstrate heterologous production of an impressive variety of small-molecule natural products and protein-based therapeutics.[4,60–62] Recapitulation of natural product pathways from plants in a eukaryotic host such as yeast is further advantageous under the consideration of spatial organization of the pathway. Many natural product pathways evolved in the context of highly specialized organelles, cells, or tissues.[63] In some cases, pathway compartmentalization may have been necessitated in order to sequester reactive biosynthetic intermediates from endogenous metabolism.[47,48] From advances in synthetic biology, targeted sub-cellular localization is possible through the use of organelle-targeting peptide signals fused to the N-terminus of pathway enzymes, or the use of intracellular protein scaffolds.[64–66] Production of tropane alkaloids in yeast required extensive localization across six sub-cellular locations.[4] In this regard, the spatial organization of the pathway is analogous to discrete process units in a factory. Similarly, full optimization requires detailed engineering approaches at each unit operation.

It is important to consider the primary metabolite building blocks required for construction of the secondary metabolite to be produced. Individual organisms exhibit variable fluxes towards given metabolic pools, dictating initial maximum titers prior to strain engineering. For example, biosynthesis of terpene products competes with primary membrane lipid metabolism, there is a

finite limit of lipid building blocks that yeast can afford to push towards a biosynthetic pathway. To address this limitation, “metabolic chassis strains” – strains with increased flux towards dedicated natural product building blocks – have been developed.[67–69]

One final advantage of yeast as a chassis for natural product production is the versatility in protein expression systems and tools and the genetic tractability to implement them. Most titer optimization efforts begin expression regulation at the level of overexpression of exogenous pathway genes and knockouts of endogenous genes to divert flux towards the desired compound.[68,70] This method is most effective in small biosynthetic pathways whose overexpression would impart little metabolic stress on the organism. Larger, more complex pathways will require more sophisticated and precise regulation tools to balance cellular fitness and expression.[71] The most obvious target for fine-tuned expression optimization is the promoter region. While improved gene expression may not always result in improved enzymatic activity, there are many processes that have seen improvement in titers from optimized expression through promoter refactoring and balancing.[72] Fine-tuning expression requires a multitude of synthetic biology tools ranging from simple small-molecule regulators to extensive genetic circuits with logic operators.[71] CRISPR dCa9-guided regulation has also been used to control gene expression with moderate sensitivity.[73,74]

When it comes to natural product biosynthesis, some hosts have obvious advantages over others. Recapitulation of complex pathways, especially those from plants, necessitates a host that has multifaceted compatibility ranging from spatial organization to expression systems. Yeast has been continually demonstrated to be an ideal host. However, the ongoing challenge for yeast platforms is to improve titers and reduce costs sufficiently to compete with traditional production methods. General strategies range from improving flux through pathway bottlenecks to ameliorating growth defects from metabolic burden or toxicity, however, a more nuanced

engineering approach may be required to extend developments of small molecule production in yeast to an industrial scale.

2. DEVELOPMENT OF A STRICTOSIDINE PLATFORM STRAIN

Strictosidine, the common precursor to thousands of MIAs, has already been produced in yeast, albeit with low titers (~0.5 mg/L). For production of complex MIAs in yeast to be viable and provide an alternative pipeline from current methods of production, strictosidine titer must be improved at least 50 to 100-fold. Such a titer could help account for unpredictable inefficiencies in the biosynthetic pathway downstream strictosidine and still result in therapeutically relevant yields of bioactive MIAs. Toward this goal, we began our work with a strain previously developed by our group, yJB051 (Table 1).[75] The yeast host yJB051 was selected as the starting point for metabolic engineering, itself modified from JHY651, which has improved respiratory growth and mitochondrial stability.[76] The strain yJB051 contains additional mutations that minimize the shunt product formation from geraniol to nepetalactol. These include deletion of two old-yellow enzymes (OYE2 and OYE3), two medium-chain dehydrogenases/reductases (ADH6 and ADH7), and one short-chain dehydrogenase/reductase (ARI1).

Table 1. Yeast Strains and Plasmids Used in This Study

strain	genotype
JHY651	BY4742; MATα prb1Δ pep4Δ his3Δ leu2Δ ura3Δ lys2Δ
yJB051	*JHY651; oye2Δ oye3Δ ari1Δ adh7Δ adh6Δ*
yJM009	yJB051; ***oye3Δ::***P_{ADH2}-CPR-T_{PRM9}, P_{PCK1}-CYB5-T_{SPG5}, P_{ICL1}-CYPADH-T_{CYC1}
yJM010	yJB051; ***oye3Δ::***P_{TEF1}-CPR-T_{PRM9}, P_{PGK1}-CYB5-T_{SPG5}, P_{TDH3}-CYPADH-T_{CYC1}
yJM025	yJM010; yprcty***1-2Δ***::P_{ICL1}-7DLGT-T_{IDP1}, P_{PCK1}-LAMT-T_{CPS1}, P_{bay_ADH2}-STR-T_{ADH1}
yJM038	yJM025; ***his3Δ::***P_{ADH2}-IO-T_{SPG5}, P_{ICL1}-7DLH-T_{PRM9}, P_{PCK1}-SLS-T_{CPS1}
yJM050	yJM025; ***ydr514cΔ::***P_{ADH2}-IO-T_{SPG5}, P_{ICL1}-7DLH-T_{PRM9}, P_{PCK1}-SLS-T_{CPS1}
yJM053	yJM050; ***iai11Δ::***P_{ADH2}-GOR-T_{PRM9}, P_{PCK1}-ISY-T_{CPS1}, P_{MLS1}-MLPL-T_{SPG5}
yRY010	yJM053; ***atf1Δ::***P_{ADH2}-G8H-T_{CPS1}
yRY017	yRY010; ***yor1Δ::***P_{ADH2}-G8H-T_{CPS1}
plasmid	description
pJB031	2μ yeast ori; URA3; ColE1 ori; AmpR
pJB040	2μ yeast ori; HIS3; ColE1 ori; AmpR; P_{ADH2}-7DLH-T_{PRM9}, P_{PCK1}-LAMT-T_{CPS1}, P_{MLS1}-SLS-T_{SPG5}; P_{ICL1}-STR-T_{IDP1}

pJB041	2μ yeast ori; URA3; ColE1 ori; AmpR; P_{ADH2}-CPR-T_{PRM9}, P_{PCK1}-CYB5-T_{SPG5}, P_{ICL1}-CYPADH-T_{CYC1}
pJB082	2μ yeast ori; URA3; ColE1 ori; AmpR; P_{ADH2}-TDC-T_{PRM9}
pJB152	2μ yeast ori; URA3; ColE1 ori; AmpR; P_{ADH2}-IO-T_{PRM9}, P_{ICL1}-7DLGT-T_{IDP1}
pJB154	2μ yeast ori; URA3; ColE1 ori; AmpR; P_{ADH2}-CPR-T_{PRM9}
pJB155	2μ yeast ori; URA3; ColE1 ori; AmpR; P_{ADH2}-CYB5-T_{PRM9}
pJB156	2μ yeast ori; URA3; ColE1 ori; AmpR; P_{ADH2}-CPR-T_{PRM9}, P_{PCK1}-CYB5-T_{SPG5}
pJB157	2μ yeast ori; URA3; ColE1 ori; AmpR; P_{ADH2}-IO-T_{SPG5}, P_{ICL1}-7DLGT-T_{IDP1}, P_{MLS1}-7DLH-T_{PRM9}
pJB158	2μ yeast ori; URA3; ColE1 ori; AmpR; P_{ADH2}-IO-T_{SPG5}, P_{ICL1}-7DLGT-T_{IDP1}, P_{MLS1}-7DLH-T_{PRM9}; P_{PCK1}-LAMT-T_{CPS1}
pJB204	2μ yeast ori; URA3; ColE1 ori; AmpR; P_{ADH2}-GOR-T_{PRM9}, P_{PCK1}-ISY-T_{CPS1}, P_{MLS1}-MLPL-T_{SPG5}; P_{ADH2}-G8H-T_{IDP1}
pJM020	2μ yeast ori; URA3; ColE1 ori; AmpR; P_{ADH2}-IO-T_{PRM9}, P_{ICL1}-7DLGT-T_{IDP1}, P_{MLS1}-GPH1-T_{CPS1}
pJM021	2μ yeast ori; URA3; ColE1 ori; AmpR; P_{ADH2}-IO-T_{PRM9}, P_{ICL1}-7DLGT-T_{IDP1}, P_{MLS1}-UGP1-T_{CPS1}
pJM022	2μ yeast ori; URA3; ColE1 ori; AmpR; P_{ADH2}-IO-T_{SPG5}, P_{ICL1}-7DLGT-T_{IDP1}, P_{MLS1}-Ca565-T_{PRM9}
pJM023	2μ yeast ori; URA3; ColE1 ori; AmpR; P_{ADH2}-IO-T_{SPG5}, P_{ICL1}-7DLGT-T_{IDP1}, P_{MLS1}-Ca610-T_{PRM9}
pJM030	2μ yeast ori; URA3; ColE1 ori; AmpR; P_{ADH2}-IO-T_{SPG5}, P_{ICL1}-7DLH-T_{PRM9}, P_{PCK1}-SLS-T_{CPS1}
pJM033	2μ yeast ori; URA3; ColE1 ori; AmpR; P_{ADH2}-IO-T_{SPG5}, P_{ICL1}-7DLGT-T_{IDP1}, P_{MLS1}-Lj7DLH-T_{PRM9}
pJM034	2μ yeast ori; URA3; ColE1 ori; AmpR; P_{ADH2}-IO-T_{SPG5}, P_{ICL1}-7DLGT-T_{IDP1}, P_{MLS1}-Rs7DLH-T_{PRM9}
pJM035	2μ yeast ori; URA3; ColE1 ori; AmpR; P_{ADH2}-IO-T_{SPG5}, P_{ICL1}-7DLGT-T_{IDP1}, P_{MLS1}-Ti17-7DLH-T_{PRM9}
pJM036	2μ yeast ori; URA3; ColE1 ori; AmpR; P_{ADH2}-IO-T_{SPG5}, P_{ICL1}-7DLGT-T_{IDP1}, P_{MLS1}-Ti18-7DLH-T_{PRM9}
pJM037	2μ yeast ori; URA3; ColE1 ori; AmpR; P_{ADH2}-IO-T_{SPG5}, P_{ICL1}-7DLGT-T_{IDP1}, P_{MLS1}-Ug7DLH-T_{PRM9}
pJM057	CEN/ARS yeast ori; HIS3; ColE1 ori; AmpR; P_{ADH2}-IO-T_{SPG5}, P_{ICL1}-7DLH-T_{PRM9}, P_{PCK1}-SLS- T_{CPS1}
pJM061	CEN/ARS yeast ori; HIS3; ColE1 ori; AmpR; P_{ADH2}-IO-T_{SPG5}
pJM062	CEN/ARS yeast ori; HIS3; ColE1 ori; AmpR; P_{ICL1}-7DLH-T_{PRM9}
pJM063	CEN/ARS yeast ori; HIS3; ColE1 ori; AmpR; P_{PCK1}-SLS- T_{CPS1}
pJM064	2μ yeast ori; URA3; ColE1 ori; AmpR; P_{ADH2}-IO-T_{SPG5}, P_{ICL1}-7DLH-T_{PRM9}
pJM065	2μ yeast ori; URA3; ColE1 ori; AmpR; P_{ADH2}-IO-T_{SPG5}, P_{PCK1}-SLS-T_{CPS1}
pJM066	2μ yeast ori; URA3; ColE1 ori; AmpR; P_{ICL1}-7DLH-T_{PRM9}, P_{PCK1}-SLS-T_{CPS1}
pJM087	2μ yeast ori; HIS3; ColE1 ori; AmpR; P_{ADH2}-GOR-T_{PRM9}, P_{PCK1}-ISY-T_{CPS1}, P_{MLS1}-MLPL-T_{SPG5}; P_{ADH2}-G8H-T_{IDP1}
pJM130	2μ yeast ori; URA3; ColE1 ori; AmpR;
pVS5	2μ yeast ori; URA3; ColE1 ori; AmpR; P_{ADH2}-G8H-T_{CPS1}

2.1. Selection of Heterologous Gene Expression System

While production of strictosidine in yeast has already been demonstrated, this strain was marred by poor growth and low production.[8] The poor growth of the strain is likely attributed to the metabolic burden imparted by heterologous expression of over 21 genes under constitutive promoters. These constitutive promoters, including THD3, TEF1, and more, are active during all yeast growth cycles and utilize cellular resources that can stunt growth. Toward development of a more robust yeast platform, we wanted to decouple the growth and production phases, allowing the yeast to grow to high density before production of our desired products began.

Crabtree-positive yeast, such as *S. cerevisiae*, exhibit a natural separation in growth cycles known as diauxic shift. Following depletion of glucose as it is fermented into ethanol, yeast will undergo a predictable shift in metabolism to aerobically oxidize ethanol via the Krebs cycle and oxidative phosphorylation. A system of promoters, which we will refer to as “ADH2 promoters,” are automatically induced following diauxic shift. A study demonstrated expression levels of green-fluorescent protein (GFP) under ADH2 promoters can reach several orders of magnitude above constitutive promoters during auto-induction in yeast.[76]

Based on these characteristics, ADH2 promoters were selected as part of our expression system for strictosidine biosynthetic pathway genes. Specifically, the promoters, ADH2p, PCK1p, ICL1p, MLS2p, and an ADH2p homolog from *Saccharomyces bayanus,* were used for all subsequent pathway gene expression experiments.

2.2 Optimizing the Expression of Pathway Accessory Enzymes

We first modified yJB051 to support the expression of four cytochrome P450 enzymes required in the strictosidine pathway (Figure 2). Functional expression of plant P450s in yeast is a challenging task and is often the limiting step for efficient pathway reconstitution. P450 enzymes require electron shuttling from redox partner enzymes to reduce the heme-bound iron after substrate oxidation for catalytic turnover.[72,77] Three *C. roseus* P450 accessory enzymes were chosen to be integrated into the yeast genome. These are the cytochrome P450 reductase (CPR), cytochrome b5 (CYB5), and a putative alcohol dehydrogenase, CYPADH. While the CPR and CYB5 are responsible for electron transfer, the CYPADH was proposed to specifically improve the function of IO, which oxidizes nepetalactol to 7-deoxyloganetic acid (Figure 2).[8] While these enzymes were used by Brown et al., in the first demonstration of strictosidine biosynthesis in yeast, the impacts of the expression profile on P450 function, metabolic flux, and strain health were not investigated. To clarify this, we established a reporter system in which the oxidation of fed nepetalactol by expressed IO serves as a proxy for the accessory enzyme function. Expression of IO alone in yeast did not accumulate any detectable 7-deoxyloganetic acid, likely due to the rapid unraveling of the hemiacetal connected to the α,β–unsaturated carboxylic acid. Coexpression of IO and 7DLGT, however, led to formation of 7-deoxyloganic acid as confirmed by comparison to an authentic standard (Figure 4B). This confirms that the glucosylation of the hemiacetal is protective and enables assessment of IO activities through quantification of 7-deoxyloganic acid by liquid chromatography/mass spectrometry (LC/MS).

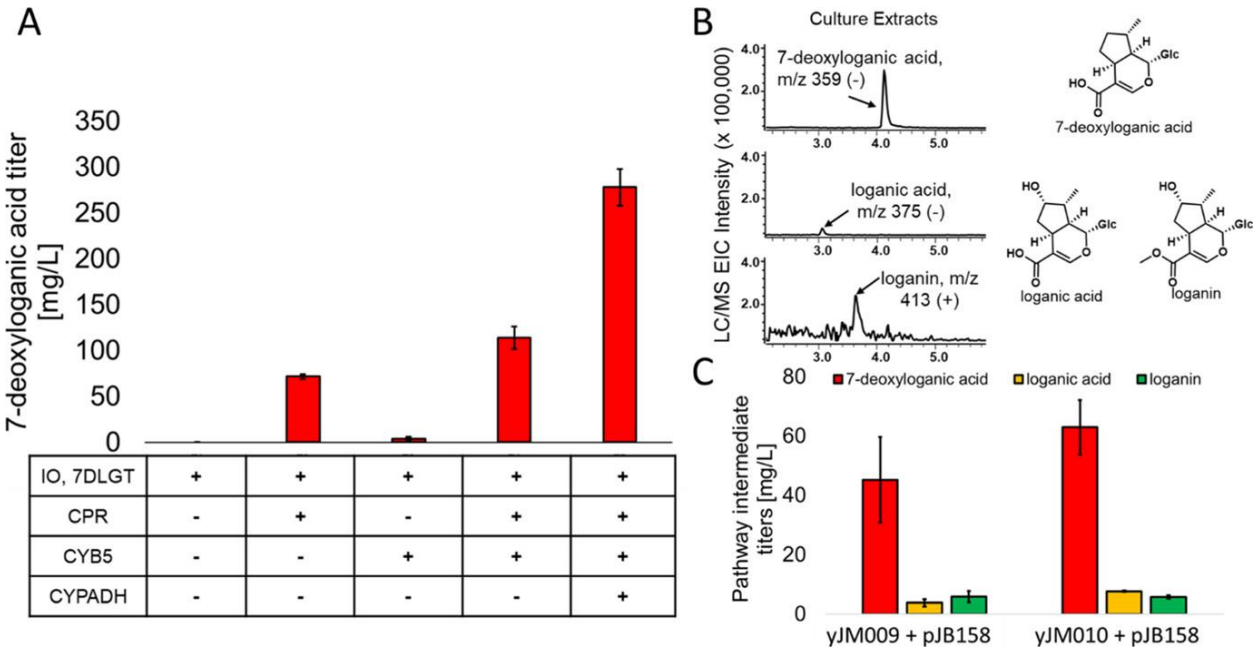


Figure 4. Optimizing expression of pathway accessory enzymes. (A) 7-Deoxyloganic acid production titers between strains expressing plasmids harboring different combinations of accessory enzymes; (B) extracted ion chromatograms of pathway intermediates of their characteristic m/z signals from LC/MS and their structures. The retention times match the standards; (C) production titers of 7-deoxyloganic acid, loganic acid, and loganin in yJM009 and yJM010 cotransformed with pJB152 and pJB040. Bars indicate the mean of biological triplicates with the error bars representing the standard error.

The strain yJB051 was transformed with a high-copy (2μ origin of replication) plasmid (pJB152, Table 1) encoding IO and 7DLGT under the control of ADH2p and ICL1p, respectively. Separate 2μ vectors containing either CPR, CYB5, CPR/CYB5, or CPR/CYB5/CYPADH under ADH2-like auto-inducible promoters were cotransformed (pJB154, pJB155, pJB156, or pJB041, Table 1). Twenty-four hours after outgrowth of the yeast transformants, the cells were inoculated in yeast extract peptone dextrose (YPD)-rich media. Nepetalactol dissolved in ethanol was added to a concentration of 336.5 mg/L to each culture and allowed to grow for a further 24 hours. The cultures were then extracted and analyzed by LC/MS for 7-deoxyloganic acid titers (Figure 4A). No production of 7-deoxyloganic acid was detected when the accessory enzymes were excluded, confirming that endogenous yeast redox partner enzymes are not compatible with IO (Figure 4A). Expression of CPR alone resulted in a 7-deoxyloganic acid titer of 71.7 ± 2.5 mg/L, while expression of CYB5 alone resulted in a much lower titer of 3.9 ± 2.3 mg/L. When CPR and CYB5 were expressed together, we observed a titer of 114.0 ± 12.5 mg/L. These results indicate that

CPR is the major electron donor to IO and can synergize with CYB5 to give the highest conversion. This is consistent with results from other researchers working with plant P450s.[7,78] When CYPADH was coexpressed, we observed a 2.5-fold increase in the 7-deoxyloganic acid titer to 278.0 ± 19.8 mg/L, in agreement with its ancillary role in the oxidation of nepetalactol.[8]

Based on these results, we next integrated a cassette encoding the accessory enzymes under the regulation of ADH2-like promoters into yJB051 at the OYE3 locus to generate strain yJM009 (Table 1). This genomic site was selected based on RNA-Seq analysis that the OYE3 locus is upregulated in the presence of the early strictosidine pathway terpene intermediates (data not shown). We hypothesized that upon addition of terpene substrate, the OYE3 locus becomes more accessible to transcriptional machinery and allows for stronger transcription. This strain was transformed with the 2μ plasmid pJB158 expressing four downstream enzymes from nepetalactol, IO, 7DLGT, 7DLH, and LAMT, each under auto-inducible promoters (pJB158, Table 1). Upon feeding nepetalactol to a concentration of 336.5 mg/L and further incubation for 24 hours, the metabolites were extracted and analyzed. We detected emergence of three expected pathway intermediates, 7-deoxyloganic acid (45.2 ± 14.4 mg/L), loganic acid (3.8 ± 1.3 mg/L), and loganin (5.9 ± 1.8 mg/L), based on comparison of mass and retention times to authentic standards (Figures 4B,C, S10, and S11).

While auto-inducible promoters were selected for expression of the biosynthetic enzymes, constitutive expression of CPR/CYB5/CYPADH to accumulate these accessory enzymes prior to P450 enzyme expression may lead to enhanced substrate turnover. To examine this possibility, we next constructed the strain yJM010. This strain contains CPR, CYB5, and CYPADH under the constitutive promoters TEF1p, PGK1p, and TDH3p, respectively. These promoters were selected as they each exhibit moderate constitutive expression levels. The strain yJM010 was transformed with pJB158 and fed nepetalactol 24 hours after inoculation into rich media, the time point at which expression of the pathway enzymes under the ADH2-like promoters is maximized. Following

metabolite extraction and analysis, pathway intermediates were quantified to 63.0 ± 4.5 mg/L of 7-deoxyloganic acid, 7.7 ± 1.0 mg/L of loganic acid, and 5.8 ± 0.4 mg/L of loganin (Figure 4C). While the loganin titer in yJM010 was similar to that of yJM009 (5.9 ± 1.8 and 5.8 ± 0.4 mg/L, respectively), 7-deoxyloganic acid and loganic acid titers were higher in yJM010, indicating an overall increase in total downstream pathway flux from the initial substrate nepetalactol. Based on these titer improvements, strain yJM010 was selected for further platform construction.

2.3. Biosynthesis of Strictosidine from Nepetalactol

Following optimization of the P450 partner enzymes, we introduced the remaining biosynthetic genes in the strictosidine pathway to establish a baseline of strictosidine production from nepetalactol. The strain yJM010 was transformed with 2μ vectors, pJB152 expressing IO, 7DLGT, 7DLH, and LAMT, and pJB040 expressing SLS and STR. All genes are under the control of ADH2-like promoters (Table 1). After strain outgrowth, nepetalactol and tryptamine both dissolved in ethanol were supplied to concentrations of 336.5 and 320.4 mg/L and the strains were further grown for 24 hours. LC/MS analysis of extracts showed the emergence of a new compound with m/z = 531. The compound was compared with an authentic standard of strictosidine obtained via chemical synthesis, (15) which showed identical retention time and MS/MS fragmentation patterns (Figure S3). Using the authentic strictosidine to establish a standard curve, the titer from the yeast pathway was measured to be 15.2 ± 1.6 mg/L between biological triplicates (Figures 5A and S12). In this strain, pathway intermediates 7-deoxyloganic acid, loganic acid, and loganin accumulated to titers of 43.9 ± 3.1, 5.2 ± 0.4, and 3.1 ± 0.6 mg/L, respectively. The molar ratios of these intermediates with respect to each other were consistent with previous strains.

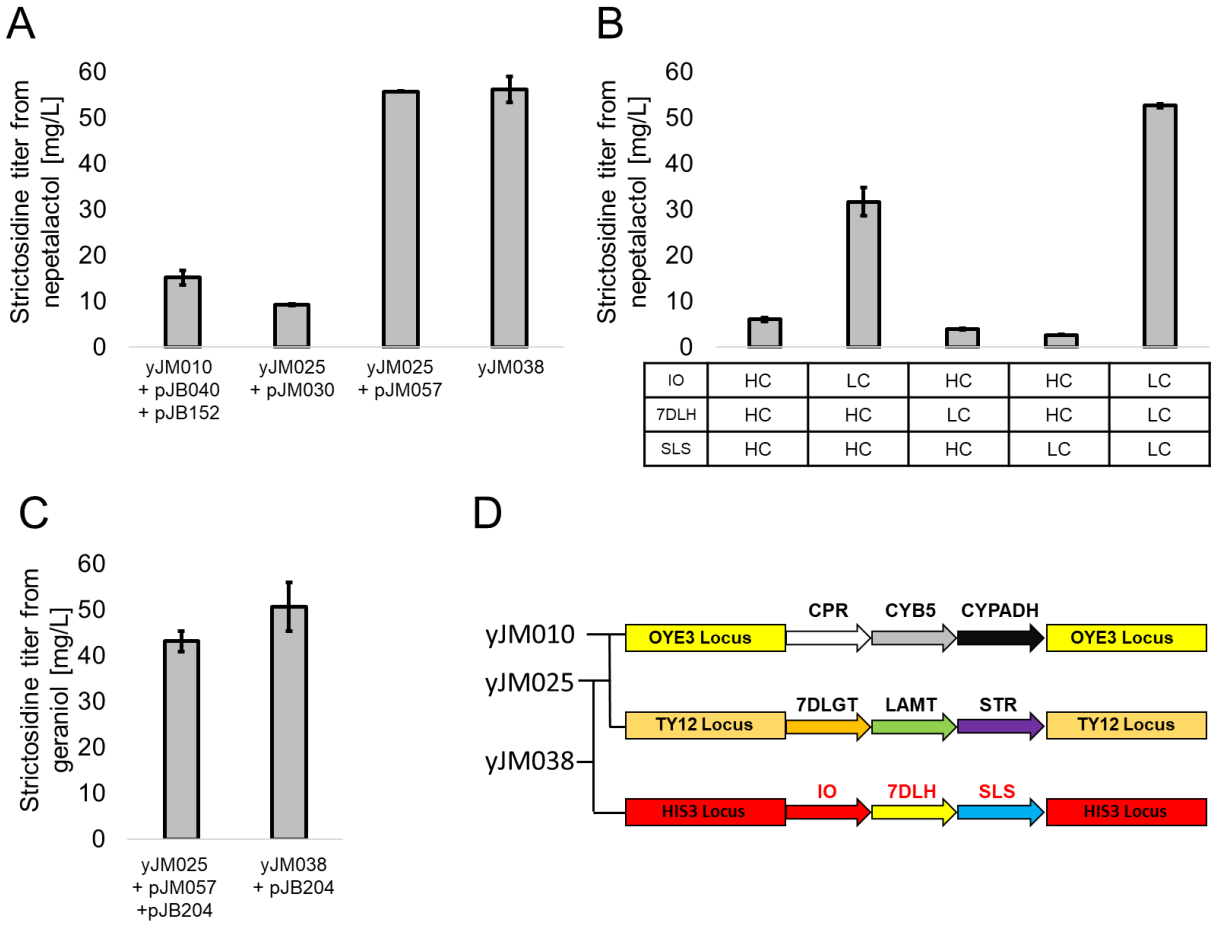


Figure 5. Comparison of strictosidine platforms. (A) Strictosidine titers of platform strains starting from nepetalactol; (B) strictosidine titers from varied copy numbers of plasmids expressing pathway P450s. HC: high-copy vector and LC: low-copy vector; (C) strictosidine titers of platform strains starting from geraniol; (D) genotypes of platform strains. Bars indicate the mean of biological triplicates with the error bars representing the standard error.

2.4. Tuning P450 Gene Copy Numbers

Because most plant P450 enzymes are translocated to the endoplasmic reticulum (ER), overexpression of these enzymes can disrupt yeast endomembrane homeostasis and activate the unfolded protein response pathway, resulting in degradation of the exogenous protein.[79,80] The effect of P450 expression levels (high copy vs low copy) on product titer, however, varies with different pathways.[81,82] High-copy (2μ) and low-copy (CEN/ARS) expression vectors containing the pathway P450s (IO, 7DLH, and SLS) were compared to evaluate changes in the strictosidine titer. The 2μ origin of replication of pJM030 was swapped with a CEN/ARS sequence to generate

plasmid pJM057 (Table 1). The low-copy pJM057 was then transformed into yJM025, and the resulting titer was measured. Remarkably, the strictosidine titer was significantly elevated to 55.8 ± 0.1 mg/L upon feeding 336.5 mg/L of nepetalactol and 320.4 mg/L of tryptamine (Figure 5A). There was a corresponding decrease in pathway intermediates to 36.0 ± 3.7, 9.4 ± 0.5, and 0.9 ± 0.3 mg/L for 7-deoxyloganic acid, loganic acid, and loganin, respectively (Figure S2).

To evaluate if altering the expression level of any one of the three P450 enzymes was responsible for the significant increase in the titer, we generated plasmid pairs that contain each pathway P450 on a low-copy vector, with the other two on a high-copy vector (pJM061 + pJM066, pJM062 + pJM065, pJM063 + pJM064, Table 1). Every plasmid pair was co-transformed into yJM025, and the resulting yeast strain was assayed quantitatively for strictosidine formation (Figure 5B). From these results, decreasing the copy number of the gene encoding IO alone resulted in the greatest improvement to the strictosidine titer from 9.2 ± 0.1 to 34.8 ± 1.1 mg/L. Several possibilities may contribute to the significant increase in the titer. First, sequence analysis of IO showed that the protein has two annotated transmembrane domains, compared with 7DLH and SLS, each having only one, which suggests that IO may disrupt the ER membrane to a greater extent during translocation. Decreasing the copy number may therefore alleviate such ER disturbances. Second, as noted earlier, the product of IO, 7-deoxyloganetic acid, is unstable, which may lead to rapid degradation if the relative activity of IO is higher compared to downstream enzyme 7DLGT.

While expressing 7DLH or SLS on low-copy plasmid did not significantly affect the titer of strictosidine, it is evident that collectively placing all three P450s on low copy vectors had the most improvement (Figure 5B). Based on this finding, a cassette encoding all three P450s under auto-inducible promoters was integrated into the HIS3 locus of yJM025 to afford yJM038 (Table 1). The plasmid-free strain yJM038 produced 56.2 ± 2.8 mg/L of strictosidine from 336.5 mg/L of nepetalactol and 320.4 mg/L tryptamine 24 hours after feeding (Figure 5A).

2.5. Biosynthesis of Strictosidine from Geraniol

Given the success of nepetalactol to strictosidine biotransformation in yJM038, we next tested conversion starting from the commodity chemical geraniol. The discovery of the major latex protein-like cyclase, MLPL, from *Nepeta mussinii*[25] completes the early pathway from geraniol to nepetalactol and decreases shunt product formation after ISY reduction (Figure 2).[83] To demonstrate that geraniol can serve as a precursor, strain yJM025 was co-transformed with the CEN/ARS plasmid pJM057 expressing IO, 7DLH, and SLS; and 2μ plasmid pJB204 expressing G8H, GOR, ISY, and MLPL (Table 1). All genes are under the control of ADH2 and ADH2-like promoters. Fed-batch assays of this transformed strain were fed to a concentration of 308.5 mg/L geraniol and 320.4 mg/L of tryptamine resulting in a strictosidine titer of 43.2 ± 2.3 mg/L (Figure 3C), a comparable titer to starting from nepetalactol. Interestingly, no pathway intermediates were detected in this strain. Entering the pathway at geraniol likely results in a steadier flux of intermediates through the pathway (especially at the IO step) and reduces accumulation at bottleneck steps like 7DLGT and 7DLH. Then, yJM038 transformed with pJB204 produced 50.7 ± 5.3 mg/L of strictosidine from geraniol and tryptamine (Figure 5C). In previously developed strictosidine-producing strains, the P450 G8H was identified as a major pathway bottleneck, precluding the use of geraniol as a feedstock.[8] The tuning of the P450 accessory enzyme and elimination of shunt pathways in combination with MLPL resulted in robust metabolic flux through the early seco-iridoid pathway to nepetalactol. Hence, strictosidine can be produced at a comparable titer starting from geraniol, a considerably cheaper precursor compared to nepetalactol, using a single plasmid-carrying yeast host.

2.6. Strictosidine Platform Growth Assays

The growth rates of the engineered strains were quantitatively compared to the starting JHY651 strain to assess the impact of the modifications to yeast robustness. Both untransformed strains and plasmid-transformed strains were assayed. For the untransformed strains, the growth rates slightly decreased as more genes were integrated into the genome, as expected from the increased metabolic load (Figure 6A). However, the impact on overall cell growth was minimal with similar stationary phase OD600 values. In the single- or double-transformed yeast strains used in production of pathway intermediates and strictosidine, cellular growth rates were impaired more significantly, with a longer lag phase and a slower exponential phase (Figure 6B). However, by approximately 16 hours after inoculation, most strains had grown to a similar cell density as JHY651. The ability for all engineered strains to reach a similar cell density as JHY651 after about 24 hours highlights the usefulness of the auto-inducible promoter system to decouple the growth and production phases of yeast despite the expression of 13 heterologous enzymes.

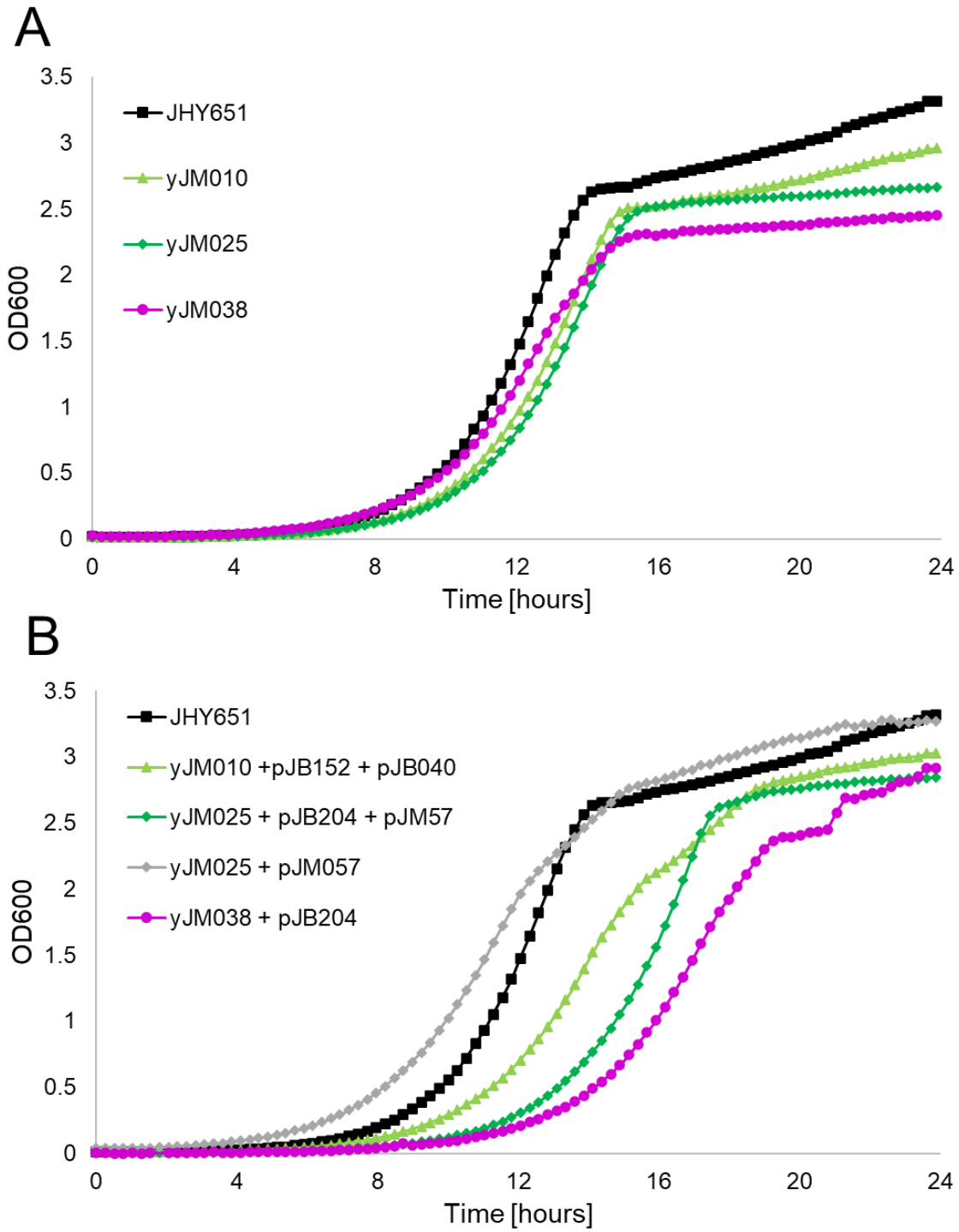


Figure 6. Comparison of strictosidine yeast strain growth rates. (A) growth curves of untransformed and plasmid-less strains compared against the wild-type; and (B) growth curves of transformed strictosidine production strains compared against the wild-type.

2.7. Purification and Characterization of Strictosidine from Yeast

To fully characterize the strictosidine produced from the strain, we scaled up (1 L) the geraniol-based production using yJM025 co-transformed with pJB204 and pJM057. The produced strictosidine was purified to homogeneity for NMR characterization. This would confirm the identity of microbial strictosidine and demonstrate feasibility in obtaining the pure compound in meaningful quantities. The yeast supernatant underwent several stages of column chromatography to arrive at fractions enriched with strictosidine. These fractions underwent final purification using semipreparative high-performance liquid chromatography (HPLC). Purified strictosidine, a yellow amorphous solid, was then analyzed by proton nuclear magnetic resonance (1H NMR), carbon nuclear magnetic resonance (13C NMR) (Figure 7), and two-dimensional NMR (Figures S4–S7). These spectra were matched to data obtained from a synthetic standard (Table 2). A nuclear overhauser effect spectroscopy experiment showed an interaction between H-3 and H-15, supporting that the strictosidine produced was the correct C3 epimer (Figure S7). Isolation of strictosidine in its pure form from yeast was made possible with the high-titer strain and underscores the usefulness of this platform in investigating downstream MIA pathways.

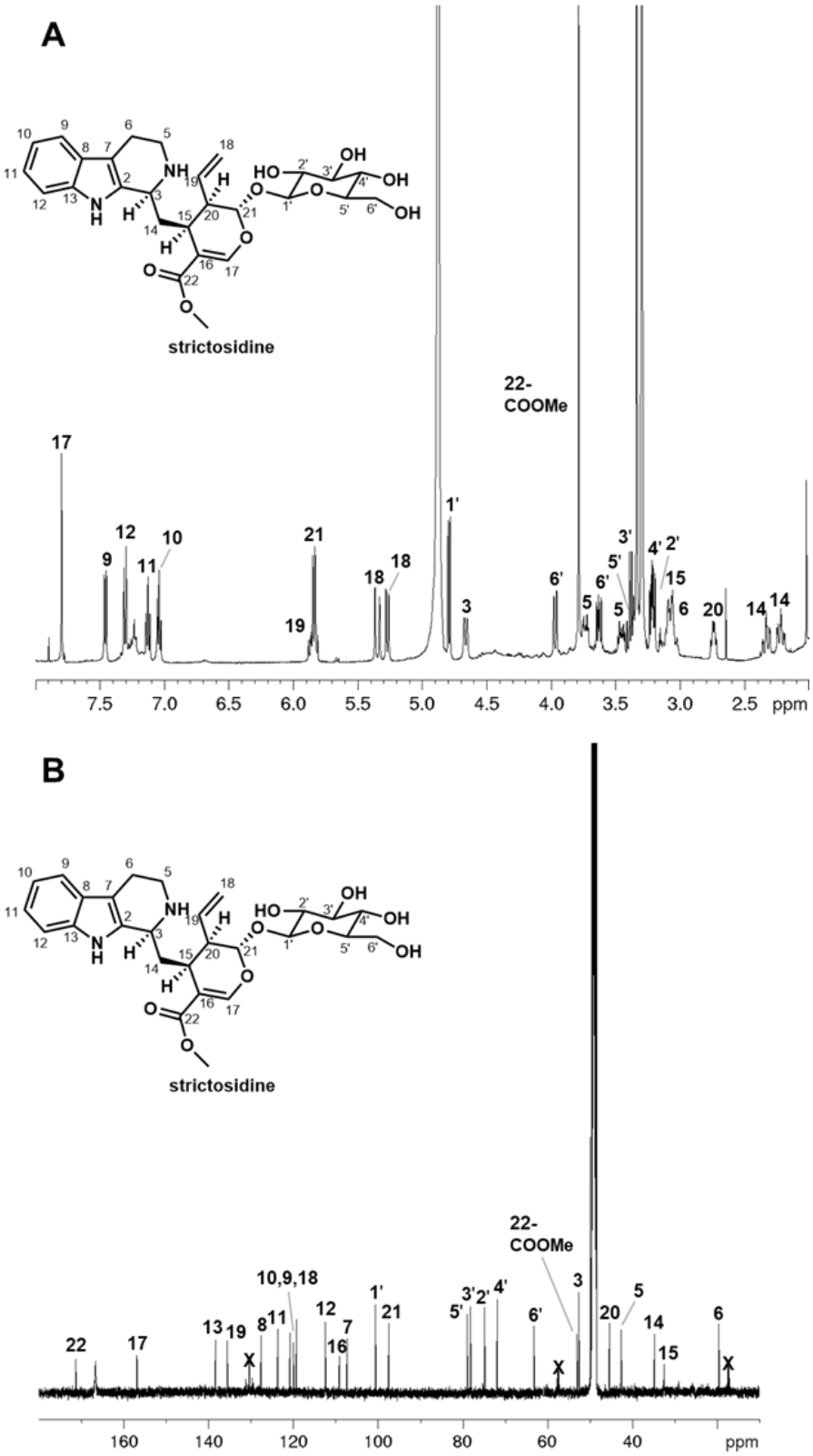


Figure 7. NMR spectra of purified strictosidine from yeast. (A) 1H NMR at 500 MHz in methanol-d4; (B) 13C NMR at 125 MHz in methanol-d4. Strictosidine is purified in the salt form as a result of acidic chromatographic conditions.

Table 2. Experimental and reported[2] ^{1}H and ^{13}C NMR data for (–)-strictosidine. The strictosidine from our experiments is in the salt form as a result of purification under acidic conditions.

	(–)-strictosidine (exp.)[a]		(–)-strictosidine (Sakamoto et al. 2020)[a]	
position	^{1}H (*J* in Hz)	^{13}C	^{1}H (*J* in Hz)	^{13}C
2	–	nd[b]	–	136.1
3	4.66, d (10.6)	52.6	4.04, d (10.5)	51.7
5	3.46, m	42.6	3.35, m	43.2
	3.74, dt, (11.3, 4.0)		3.05, dt (11.0, 4.0)	
6	3.03, m	19.6	2.75, dt (14.5, 4.0)	22.4
	3.10, m		2.85, dddd (13.5, 7.0, 4.0, 1.5)	
7	–	107.2	–	108.4
8	–	127.4	–	128.5
9	7.46, d (7.9)	119.8	7.38, d (7.5)	118.6
10	7.04, t (7.5)	120.6	6.96, td (8.0, 1.0)	119.7
11	7.13, t (7.6)	123.5	7.03, td (8.0, 1.0)	122.0
12	7.31, d (8.1)	112.3	7.25, d (8.0)	111.8
14	2.24, m	34.8	2.08, ddd (14.0, 11.0, 3.0)	37.1
	2.12, m		2.00, ddd (15.0, 11.0, 4.0)	
15	3.09, m	32.5	3.00, ddd (12.0, 9.5, 4.5)	32.7
16	–	109.0	–	110.8
17	7.70, s	156.9	7.70, s	155.3
18	5.25, d (17.4)	119.1	5.32, td (17.5, 1.5)	119.1
	5.17, d (10.6)		5.22, d (10.5)	
19	5.85, ddd (17.4, 10.6, 7.5)	135.4	5.85, ddd (18.0, 10.5, 7.5)	136.2
20	2.74, ddd (8.6, 7.5, 2.3)	45.4	2.69, ddd (12.5, 9.0, 5.5)	45.9
21	5.84, d (8.6)	97.3	5.83, d (8.5)	97.6
22	–	171.3	–	170.2
22-CO_2Me	3.75, s	53.0	3.76, s	52.1
1'	4.79, d (7.9)	100.4	4.79, d (8.0)	100.3
2'	3.22, t (7.9)	74.7	3.22, t (8.0)	74.7
3	3.39, d (9.0)	78.0	3.39, d (9.0)	78.0
4'	3.25, t (9.0)	71.7	3.25, t (9.0)	71.7
5'	3.36, m	78.8	3.36, m	78.7
6'	3.97, dd (11.8, 1.9)	63.0	3.95, dd (12.0, 2.0)	62.9
	3.63, dd (11.8, 7.0)		3.65, dd (12.0, 6.5)	

[a]Recorded at 500 MHz for ^{1}H and 125 MHz for ^{13}C in methanol-d_4. [b]Not detected.

3. OPTIMIZING PRODUCTION OF STRICTOSIDINE AND ANALOGS

Following the development of our geraniol-based strictosidine platform, we wanted to further optimize our strain to improve titer. One approach is to improve the activity of pathway enzymes, making each catalytic transformation more efficient. A straightforward method towards improved activity is to evaluate differences in activity from different homologs of the same enzyme from different species. This approach has led to markedly improved titers in several yeast systems.[9,84,85] Next, we decided to investigate some gene targets involved in primary yeast metabolism to access for effects on strictosidine and pathway intermediate titers. Finally, we tested the capacity of our platform to produce strictosidine analogs by feeding modified substrates.

3.1. Bioprospecting of 7DLH Homologs

As evident from the bioconversion of nepetalactol to loganic acid upon coexpression of IO, 7DLGT, 7DLH, and LAMT, 7-deoxyloganic acid, the product of 7DLGT, is the major product (Figure 4C). Quantifying the levels of metabolites extracted from intracellular and supernatant fractions revealed that >80% of 7-deoxyloganic acid accumulates in the culture supernatant (Figure S1). We reason that this could be due to the low activity of *C. roseus* 7DLH (Cr7DLH), which may result in most of the substrate being transported to the extracellular space by yeast endogenous transporters. To potentially improve the activity of 7DLH, we replaced the Cr7DLH in the expression plasmid with a panel of seven putative 7DLH enzymes (pJM022-pJM023 and pJM033-pJM037, Table 1) from several different plant families including *Apocynaceae, Rubiaceae, Caprifoliaceae, and Nyssaceae* (Figure S8). Sequence alignments indicate that all 7DLH homologue sequences contain a membrane anchor region at the N-termini. Alignment of CPR sequences from these species showed high sequence identity to that of the C. roseus CPR (Figure S9). Metabolite analysis showed that four of the seven bioprospected 7DLHs supported loganin production (Figure 8). Based on loganin titers, Cr7DLH remained the one with the highest

activity in yeast. 7DLH from *L. japonica* showed the next highest activity at ~82% activity relative to Cr7DLH, while 7DLH from *R. serpentina* and two 7DLH homologues *from Catharanthus acuminata* both showed less than 20% activity. The 7DLH homologues from *T. iboga* and *U. guianensis* did not support any biosynthesis of loganin in yeast, as only 7-deoxyloganic acid is detected. As a result, Cr7DLH (referred to as 7DLH) was used in all subsequent studies.

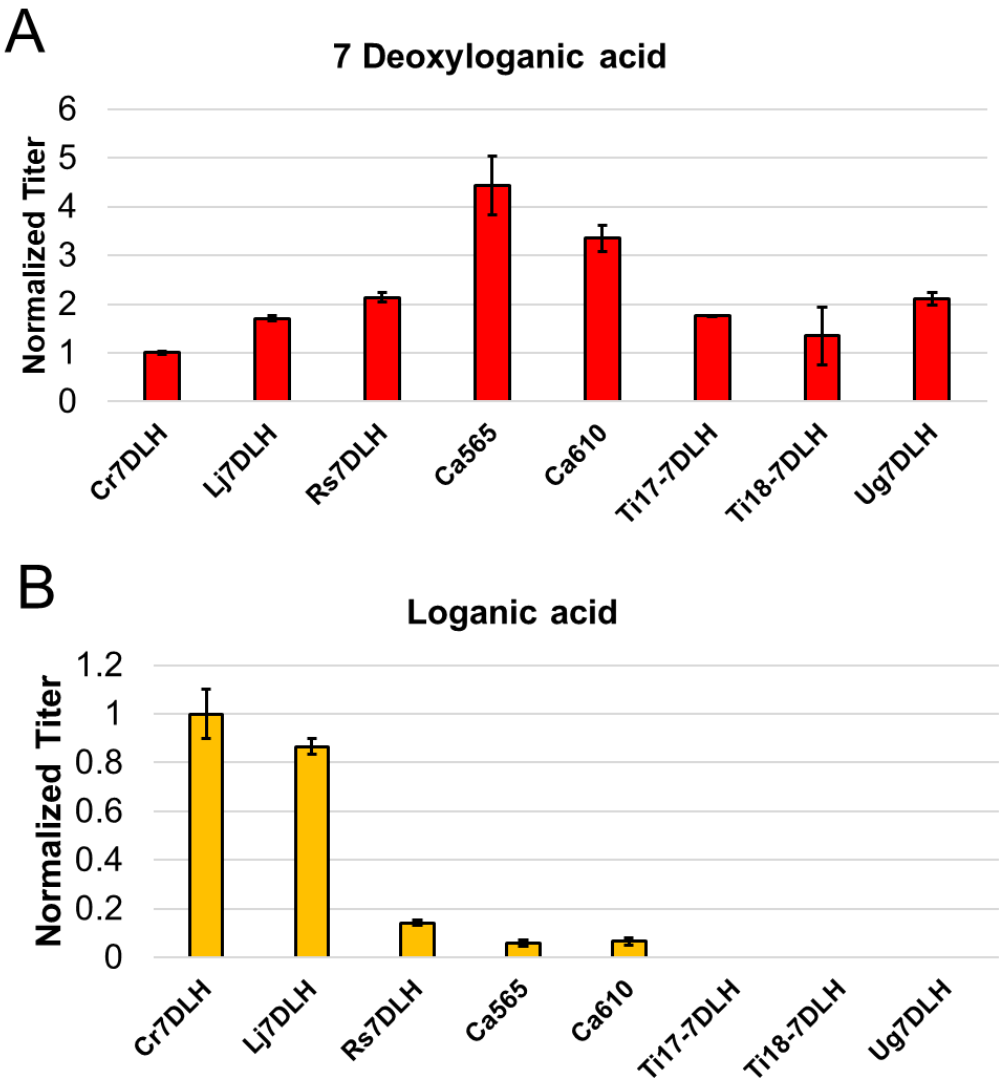


Figure 8. Bioprospecting 7DLH Enzymes for improved bioactivity. (A) Production of 7deoxyloganic acid from strains expressing different putative 7DLH enzymes. (B) Production of loganic acid from strains expressing different putative 7DLH enzymes. Titers are normalized against production using the C. roseus 7DLH. Bars indicate the mean of biological triplicates with the error bars representing the standard error.

3.2. Probing Glucosylation Machinery

Glucosylation of the iridoid scaffold catalyzed by 7DLGT serves to mask the hemiacetal and prevent spontaneous ring opening. Initial studies in our nepetalactol platform show only ~38% conversion to 7-deoxyloganic acid (Figure 4A). While this was later discovered to be largely attributed to suboptimal P450 expression, low bioavailability in the glucose in a transferable form (UDP-glucose) due to competing primary metabolism pathways could be another limiting factor.[86] Two key enzymes in the UDP-glucose pathway are GPH1 and UGP1 which catalyze the release of glucose-1-phosphate from glycogen and the formation of UDP-glucose from glucose-1-phosphate and uridine triphosphate (UTP), respectively. In tropane alkaloid biosynthesis, overexpression of UGP1 resulted in a near 2-fold increase in accumulation of the glucosylated pathway intermediate.[4] Based on this, we decided to investigate if overexpression of GPH1 and UGP1 could improve MIA intermediate titers. Yeast strain yJM010 was transformed with either 2μ plasmids pJM020, pJM021, (which contain genes encoding IO, 7DLGT, and either GPH1 or UGP1, respectively) or the control plasmid pJM152 which contains genes encoding IO and 7DLGT (Table 1). Production of 7-deoxyloganic acid from nepetalactol was evaluated through a standard fed-batch assay of biological triplicates of each of these transformants. The control strain was also alternatively fed UDP-glucose. Unfortunately, overexpression of GPH1 or UGP1 had similar or decreased 7-deoxyloganic acid titers compared to the control (Figure 9). The control strain had a titer of 225.8 ± 11.4 mg/L, while GPH1 overexpression had a titer of 205.0 ± 8.68 mg/L and UGP1 overexpression had a titer of 225.3 ± 6.1 mg/L. The culture fed UDP-glucose resulted in slightly decreased titer as well at 205.8 ± 14.6 mg/L. Together, these results could indicate that UDP-glucose is not limiting in production of 7-deoxyloganic acid, or that high-copy overexpression of these primary metabolism genes may have deleterious effects on yeast fitness or metabolism.

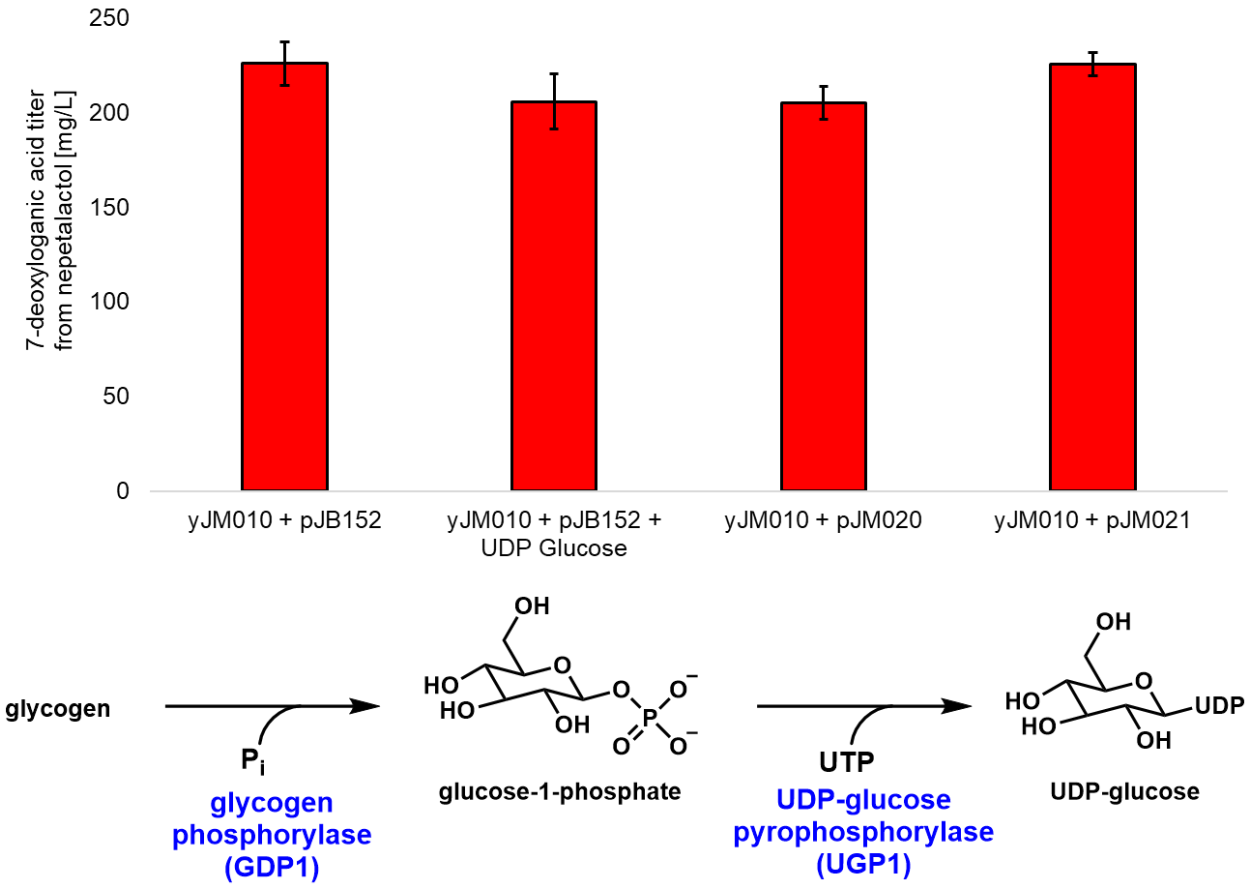


Figure 9. Overexpression of UDP-glucose pathway enzymes.

3.3. Limitations of G8H

In our geraniol-based platform, the pathway enzymes to convert geraniol to nepetalactol (G8H, GOR, ISY, and MLPL) are all expressed on a single, high-copy (2μ origin) plasmid. Towards development of a plasmid-free strain, we next integrated the non-P450 encoding genes for GOR, ISY, and MLPL into our strain at the *iai11* locus to yield strain yJM053 (Table 1). To compare how integration of those three genes impacts strictosidine titer, yJM053 was transformed with a 2μ plasmid containing G8H expressed under ADH2p, pVS5 (Table 1). yJM053 transformed with pVS5 had a strictosidine titer similar to pJM038 transformed with pJB204 (60.7 ± 4.9 mg/L and 61.2 ± 4.5 mg/L, respectively) indicating that single-copy integration of the genes encoding GOR, ISY, and MLPL is sufficient and not-limiting toward strictosidine production (Figure 10).

The final gene to be stably integrated into our yeast platform is the gene encoding the first P450 in the pathway. Based on observations by Brown *et al.* in their strictosidine study, G8H is limiting and required integration of four copies to maximize strictosidine titer.[8] This contrasts our observations with optimal expression of other pathway P450s as outlined in Section 2.4. Regardless, we decided to move forward with integration of a G8H-encoding integration cassette into yJM053 at the *atf1* locus resulting in a plasmid-free strain, yRY010. Biological triplicates of this strain were assayed following standard fed-batch procedures, with a pre-culture in rich YPD medium instead of a minimal selective media that would be used in plasmid-based strains. The resulting strictosidine titer, 6.3 ± 0.2 mg/L, was about 10-fold lower than our control strain, yJM053 transformed with G8H, with a titer of 61.2 ± mg/L. This result confirmed that a single-copy expression of G8H was not sufficient to maintain high strictosidine titer. Before attempting to integrate more copies of G8H into our strain, we wanted to investigate other potential contributions to diminished strictosidine titer.

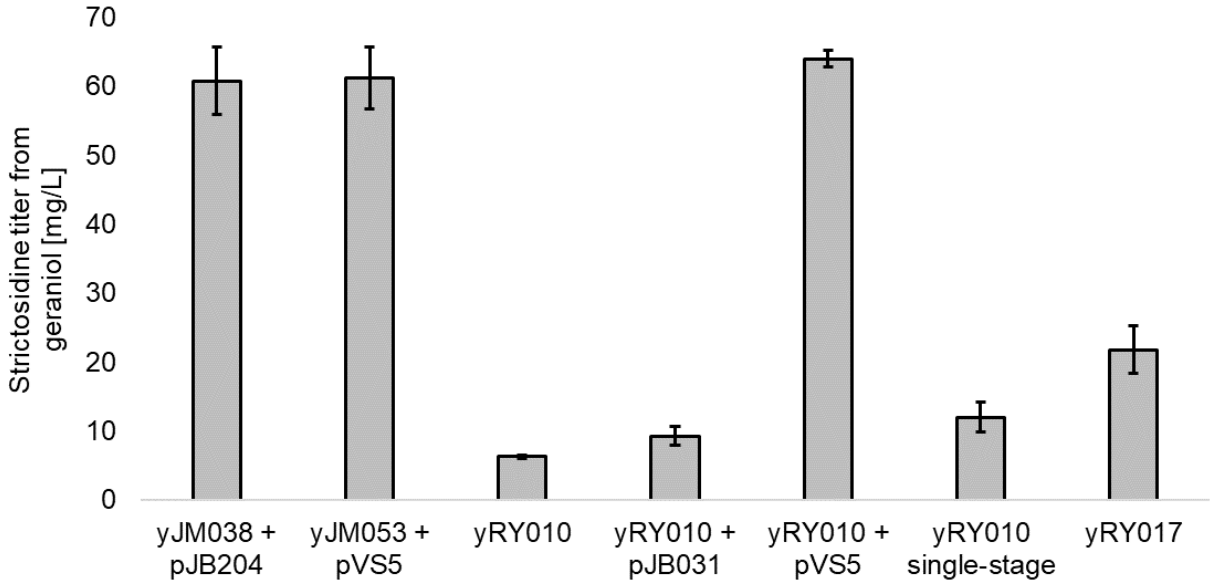


Figure 10. Building towards a plasmid-free geraniol-based platform.

One difference between these strains is the alleviation of the uracil auxotroph in yJM053 + pVS5 with expression of URA3 in the plasmid backbone. Assaying a transformation of yRY010

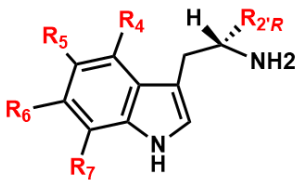
with an empty vector (pJB031, Table 1) containing the gene encoding URA3 recovered some strictosidine titer to 9.3 ± 1.4 mg/L but was not complete. Recovering G8H copy number via transformation of yRY010 with pVS5 fully restored strictosidine production to 63.9 ± 1.1 mg/L, further supporting limitations of G8H expression (Figure 10). Another difference is the requirement of a pre-culture step in the plasmid-based strains to allow for sufficient propagation of plasmid before inoculation into YPD medium for high density outgrowth. Since yRY010 does not require a plasmid, we inoculated single colonies, in biological triplicate, into 1.5 mL of YPD medium and fed 24 hours, bypassing the two-stage culturing. Interestingly, this resulted in a 2-fold increase in strictosidine titer from a two-stage culture of yRY010, 6.3 ± 0.2 to 12.0 ± 2.1 mg/L (Figure 10). It is unclear why this process change results in improved titer. There is no difference in OD600 values between the single- and two-stage cultures, precluding the possibility of higher cell density (and thus concentration of pathway enzymes relative to fed substrate) attributing to increased strictosidine production. Regardless, for any subsequent assays with plasmid-free strains, we will incorporate a single-stage culture process for the best production.

Finally, we wanted to understand the relationship between an additional integrated copy of the gene encoding G8H and strictosidine titer. Gene copy-numbers do not always correlate linearly with enzyme expression or product titers. Insight into how a second integrated copy of the gene encoding G8H could improve titer could inform on diminished returns of three or more copies. A second integration cassette for expression G8H was integrated into yRY010 at the *yor1* locus, resulting in strain yRY017. Single-stage culturing fed-batch assays of yRY017 strain showed significant improvement in strictosidine titer at 21.8 ± 3.4 mg compared to yRY010 at 12.0 ± 2.1 mg/L (Figure 10). The integration of a second copy had an improvement was about 2-fold which gives us confidence that future integrations of additional copies of G8H could fully recover strictosidine production in the plasmid-based strains and provide a more stable, plasmid-free platform.

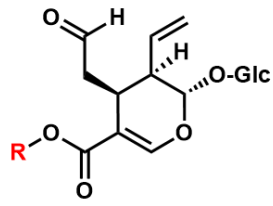
3.4. Production of Strictosidine Analogs

While some alkaloids are front-line therapeutics, others in their native forms suffer from low potency and/or perverse side-effects. Development of structural analogs of many drugs using synthetic or semi-synthetic methods has resulted in improved bioactivity and target specificity.[87] Notwithstanding these achievements, many analogs are not accessible with traditional chemical methodologies due to unstable chemical structures, inaccessible carbon centers, inability to control stereochemistry, and more.[88] The complex biochemistries and broad substrate scopes of enzymes can be leveraged to generate novel analogs that were previously inaccessible. While cell-free single pot reactions consisting of dozens of enzymes have been demonstrated, expression in a microbial host is a more straightforward approach towards accessing these analogs.[83,89]

Using the strictosidine producing yeast strain constructed as mentioned above, we next tested the ability of the strain to produce analogs of strictosidine through precursor-directed biosynthesis. In particular, STR was shown to have relaxed substrate specificity toward substituted tryptamine and secologanin analogs, with over 15 unique strictosidine analogs being accessed with unmodified STR (Figure 11).[41,42,44] Because no strictosidine production can be detected without supplementing tryptamine, feeding substituted tryptamines would lead to the biosynthesis of modified strictosidine analogues with minimal background. A similar strategy was recently demonstrated by Li et al. to generate modified noscapine analogues from substituted tyrosines.[6]



tryptamine analogs



secologanin analogs

R = ethyl, allyl, propagyl**, pentynyl**

	i – iv	v - viii	ix - x	xi - xv	xvi - xviii
R_4	H, F, Me, OMe	H	H	H	H
R_5	H	Cl, Br, F, OH	H	H	H
R_6	H	H	F, OMe	H	H
R_7	H	H	H	Cl, Br, F, Me, OMe	H
$R_{2'R}$	H	H	H	H	(-)-Me, (+)-Me, CH_2OH*

*- F232L mutant required, **-D117A mutant required

Figure 11. Substrate scope of STR with tryptamine and secologanin analogs.

A panel of five substituted tryptamines (5-bromotryptamine, 6-methoxytryptamine, 6-chlorotryptamine, 7-chlorotrypamine, and 7-fluorotryptamine) along with geraniol were fed into separate yJM025 cultures co-transformed with pJM057 and pJB204. We observed no growth defects between strains following feeding compared to the unmodified tryptamine control. Twenty-four hours after feeding, cultures were extracted with acetone and ran on QTOF-LC/MS for analysis. The chromatographs were filtered for the expected masses of the modified strictosidine products. New compounds were detected upon 7-fluorotryptamine and 7-chlorotryptamine supplementation (Figure 12B). The retention time shifts of the compounds are consistent with halogen incorporations. MS/MS analysis of the strictosidine analogues further suggested that these signals correspond to halogenated strictosidine analogues (Figure 12C). The differences between the 7-fluorostrictosidine and strictosidine parent ion (549.224 vs 531.234, respectively) and major daughter ions (532.198 and 370.145 vs 514.209 and 352.155, respectively) are 17.99 mass units, corresponding to a replacement of a hydrogen with a fluorine atom. Similarly, the differences in masses of parent and daughter ions between 7-chlorostrictosidine and strictosidine (566.197, 549.171, and 387.119 vs 531.234, 514.209, and 352.155, respectively) are 34.96 mass

units, corresponding to the replacement of a hydrogen with a chlorine atom. The lack of incorporation of other tryptamine analogues is consistent with previous reports which stated that STR does not tolerate 5- and 6-substituted tryptamines well.[43] Point mutations that result in a larger binding pocket of STR have been identified.[42] Recapitulation of these mutations in the STR gene may expand the scope of the modified strictosidine analogues obtainable from yeast-based precursor-directed biosynthesis.

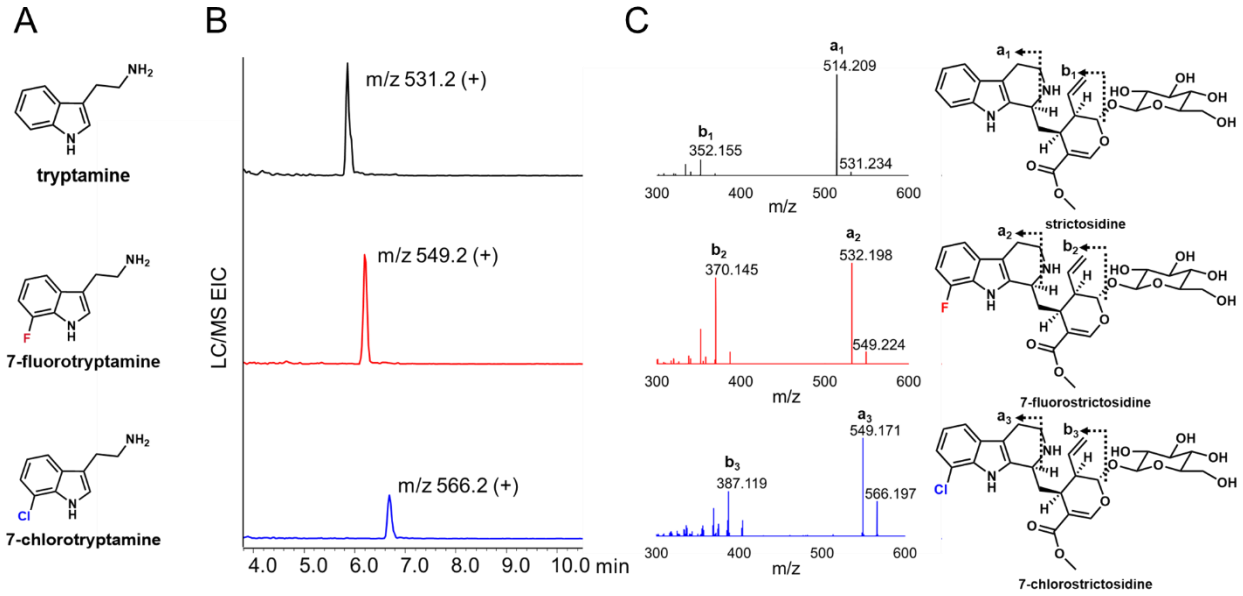


Figure 12. Production of halogenated strictosidine derivatives. (A) Structures of tryptamines successfully incorporated into strictosidine *in vivo;* (B) Extracted ion chromatogram (EIC) of characteristic *m/z* $[M+H]^+$ signal for different strictosidine analogs from LC/MS analysis; (C) Tandem mass spectrometry (MS/MS) fragmentation patterns from QTOF-LC/MS for strictosidine (black), 7-fluorostrictosidine (red), and 7-chlorostrictosidine (blue) and corresponding predominant product ion structures.

3.5. Expression of Tryptophan Decarboxylase from *C. roseus*.

As noted previously, strictosidine production in our yeast platform relies on supplementing tryptamine as tryptamine does not accumulate to detectable levels in yeast. In addition to being expressed in the original strictosidine platform work by O'Connor and co-workers, tryptophan decarboxylase (TDC) from *C. roseus* has also been expressed in yeast towards the production of potent hallucinogenic psychoactive natural product, psilocybin.[69] This gave us confidence that TDC could be efficiently expressed in our platform to generate a tryptamine-free strain. We co-expressed a plasmid containing TDC (pJB082) with pJM087 in yJM038 and assayed it following standard assay conditions. After 24 hours in rich media, the cultures were fed 308.5 mg/L geraniol and either 320.4 mg/L tryptamine in 15 uL ethanol or 15 uL of pure ethanol. The result was strictosidine titers were equal between the strains that were and were not supplemented with tryptamine (Figure 13). Further, we observed accumulation of about 50 mg/L of tryptamine in the unfed cultures. Together this indicated that the expression of TDC was sufficient and non-limiting for high-titer strictosidine production in our strain.

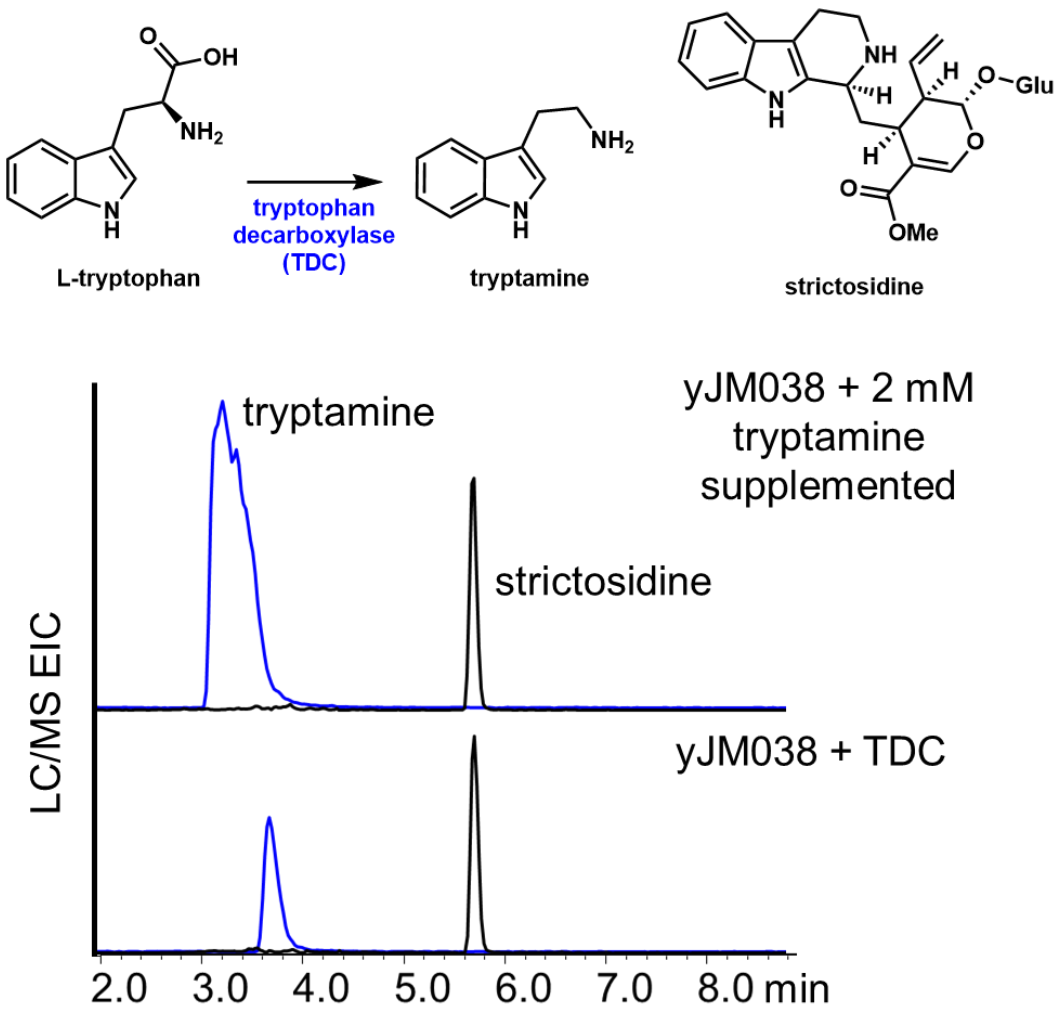


Figure 13. Expression of tryptophan decarboxylase in yeast.

4. PRODUCTION OF NOVEL MONOTERPENE INDOLE ALKALOIDS AND ANALOGS

In recent years, the biosynthetic routes to many bioactive MIAs have been elucidated, including vinblastine, ibogaine, alstonine, quinine, and mitragynine, many of which have been elucidated to completion.[18,20,22,24,27,52,90–92] A pipeline for vinblastine production in yeast was recently demonstrated by µg production of catharanthine and vindoline, two MIAs that can be condensed *ex-vivo* to afford vinblastine.[9] This gave us confidence that pathway enzymes downstream of strictosidine could be expressed well in yeast. Two bioactive MIA classes we focused on in this work are the heteroyohimbine and corynanthe-type MIAs. Alstonine and mitragynine are two MIAs from these classes, respectively, that are of particular interest for their potential therapeutic and psychoactivities.

4.1. Expression of Strictosidine β-Glucosidase

Most MIA scaffolds downstream strictosidine first rely on the deglucosylation of strictosidine by SGD to afford strictosidine aglycone. As stated previously, strictosidine aglycone exists in equilibrium in many forms. We wanted to understand the distribution of these forms by LC/MS analysis. However, since a standard of strictosidine aglycone is not readily available, we decided to generate the compound through an *in vitro* reaction with purified SGD and strictosidine. Following 1 hour incubation of 100 µM strictosidine with 50 nM SGD, we observed the appearance of 4 new peaks on LC/MS compared to a control without SGD (Figure 14). Three of these peaks had a major mass response at +351 m/z and one at +369 m/z. Comparing these peaks with m/z ratios to the structures of known forms of strictosidine aglycone, we putatively assigned the major 351 peak to cathenamine, and the major 369 peak to strictosidine aglycone (open form). One of the minor 351 peaks could correspond to the unstable dialdehyde form, 4,21-dihydrogeissoschizine. With an understanding of how strictosidine aglycone forms appear on

LC/MS, we are well positioned for targeted metabolomics for expression studies of SGD in our yeast platform strains.

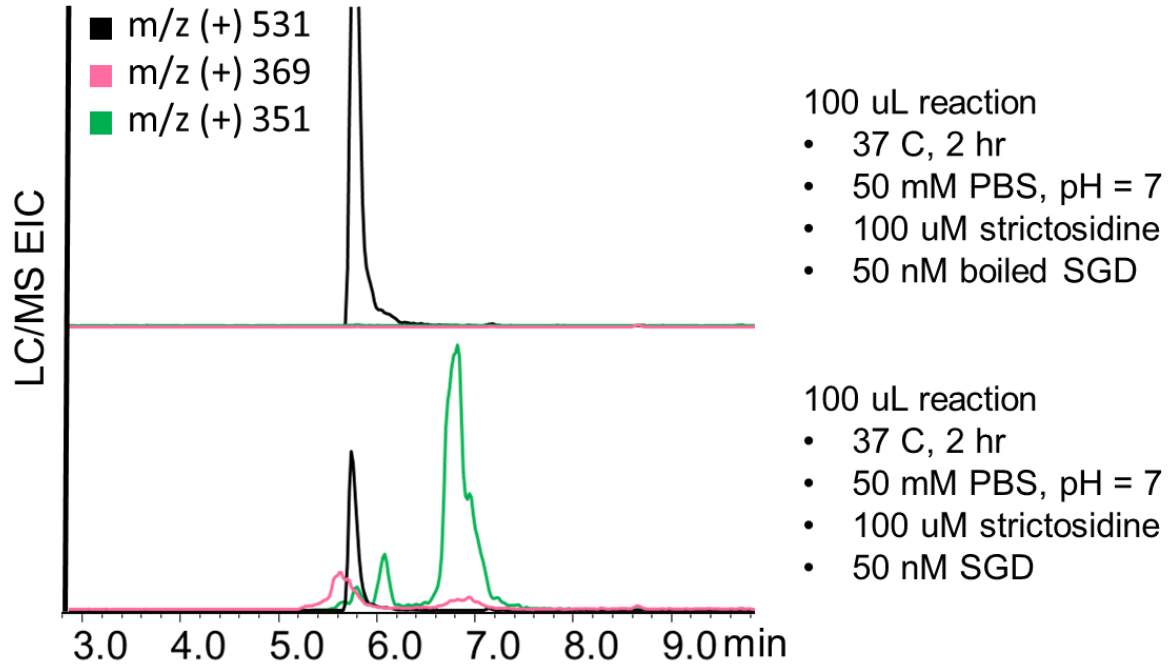


Figure 14. In vitro expression of SGD.

Our initial attempts to express SGD in yeast were unsuccessful. When expressing SGD on a plasmid in yJM038, we were unable to detect accumulation of any strictosidine aglycone forms. This prompted us to bioprospect for other SGD homologs from other species to see if any of them would be active in yeast without any modifications or engineering. We were able to obtain the sequences for four additional SGD candidates from publicly available databases. These were each cloned into yeast expression vectors under ADH2 promoters and transformed in yJM038 along with pJM087. Triplicates of each transformant were assayed according to standard fed-batch procedures with geraniol and tryptamine and analyzed by LC/MS. Careful analysis of all three major forms of strictosidine aglycone in each sample revealed only RsSGD and MsSGD had any observable activity in yeast (Figure 15). RsSGD is significantly more active than MsSGD

by about three-fold. Contrary to the *in vitro* experiment, cathenamine was not the major form observed in yeast culture extract, with more accumulation of strictosidine aglycone open form. We also observed a large accumulation of strictosidine remaining in these culture extracts, indicating that RsSGD and MsSGD, while active, are not very efficient. Low conversion could also be explained by low substrate access based on previous observations of strictosidine and other MIA pathway intermediates extracellular accumulation. Based on these findings, we decided to use RsSGD for all further studies.

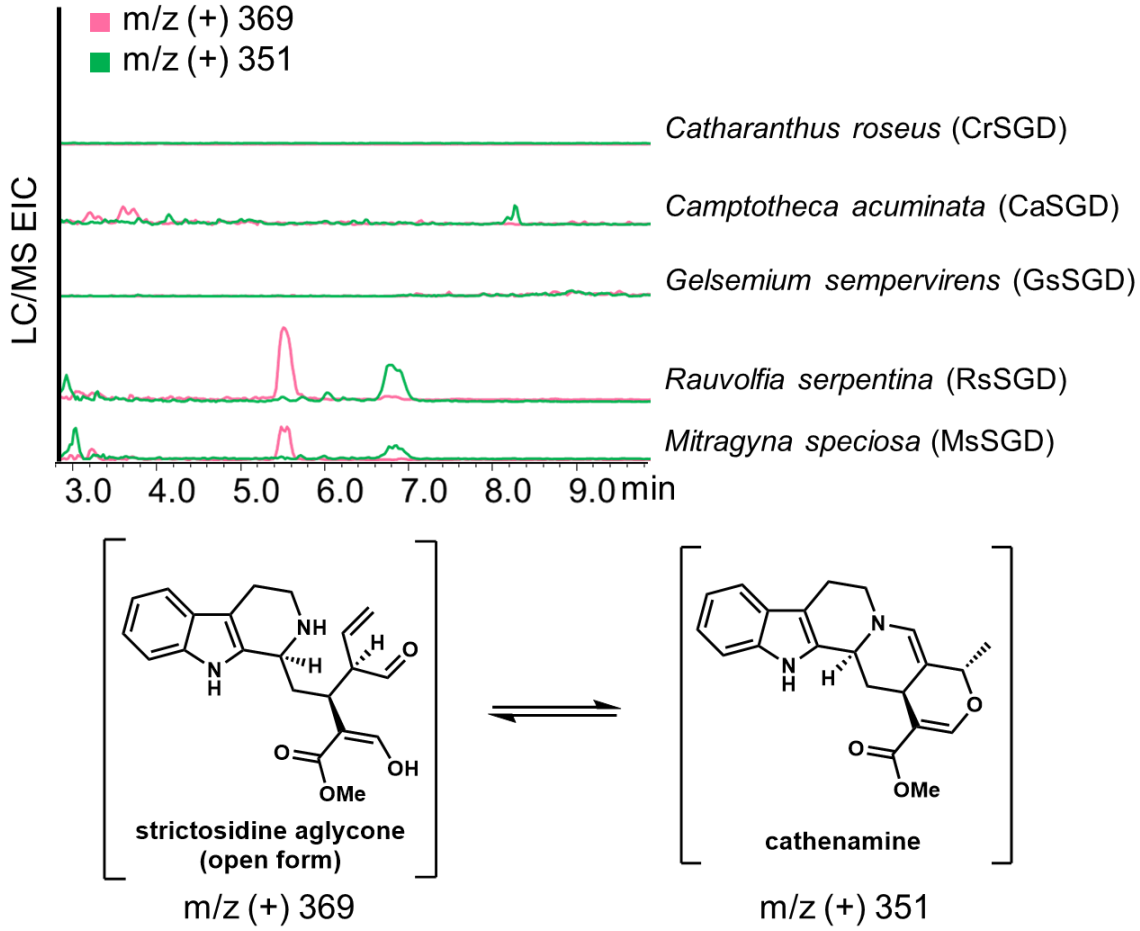


Figure 15. Bioprospecting of SGD variants.

4.2. Production of Alstonine and Analogs

Overcoming the pathway flux bottleneck at RsSGD is critical towards accessing bioactive MIAs in isolatable titers. Recently, a rational engineering approach towards improving RsSGD did not result in significant improvement in activity in yeast.[9] Directed evolution could lead to an improved variant in yeast, but such an approach necessitates a high-throughput screening method. As stated previously, the endpoint compounds in the heteroyohimbine MIA class, alstonine and its epimer serpentine are fluorescent, emitting blue light at ~420 nm. They are formed by oxidation of tetrahydroalstonine (or its epimer ajmalicine) which forms the aromatic β-carboline moiety. Towards the goal of development of a screening platform for MIA production, we wanted to reconstitute the alstonine biosynthetic pathway in yeast.

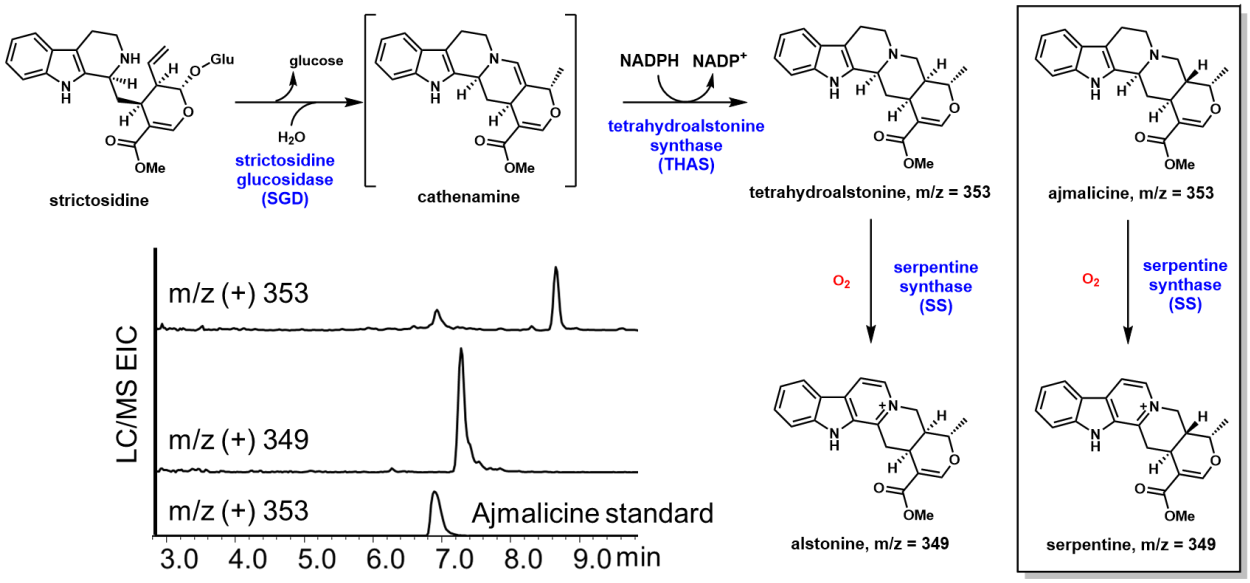


Figure 16. Biosynthetic pathway of heteroyohimbine alkaloids and production in yeast.

We introduced the biosynthetic genes in the alstonine pathway into our geraniol-based strictosidine platform to evaluate their activity in yeast. The strain yJM053 was transformed with 2μ vectors, pVS5 and pMD029 expressing RsSGD, THAS, and SS, all under AHD2 promoters (Table 1). Following standard fed-batch assay procedures single colonies of transformants, in triplicate, geraniol and tryptamine were supplied to the cultures. LC/MS analysis of extracts

showed the emergence of two new peaks with m/z = 351 and 349 (Figure 16) which are predicted to be tetrahydroalstonine and alstonine, respectively. Strictosidine and cathenamine peaks were observed as well, reaffirming the bottleneck of RsSGD incomplete conversion by THAS. A standard of the tetrahydroalstonine epimer, ajmalicine, showed a similar retention time to that of the new m/z = 351 peak, supporting that the new peak could be tetrahydroalstonine. The putative tetrahydroalstonine and alstonine peaks were quantified using an ajmalicine standard curve to ~0.5 mg/L and 3.1 mg/L, respectively.

We decided to evaluate if THAS and SS could accommodate strictosidine analogs to generate novel tetrahydroalstonine and alstonine analogs. A panel of tryptamines, 7-fluorotryptamine, 7-chlorotryptamine, and 4-methoxytryptamine, along with geraniol were fed, separately, to yJM053 co-transformed with pVS5 and pMD029. Analysis of culture extracts on LC/MS show emergence of new peaks corresponding to the predicted mass shifts from the fed tryptamine analogs (Figure 17). The culture extracts fed 7-fluorotryptamine had anew peaks of m/z = 367, the culture extracts fed 7-chlorotryptamine had a new peak of m/z = 383, and the culture extracts fed 4-methoxytryptamine had a new peak of m/z = 380. Interestingly, in this experiment, there was no accumulation of tetrahydroalstonine or its analogs in any cultures. None of these peaks were observed in the control strains, supporting that these new peaks are related to strictosidine.

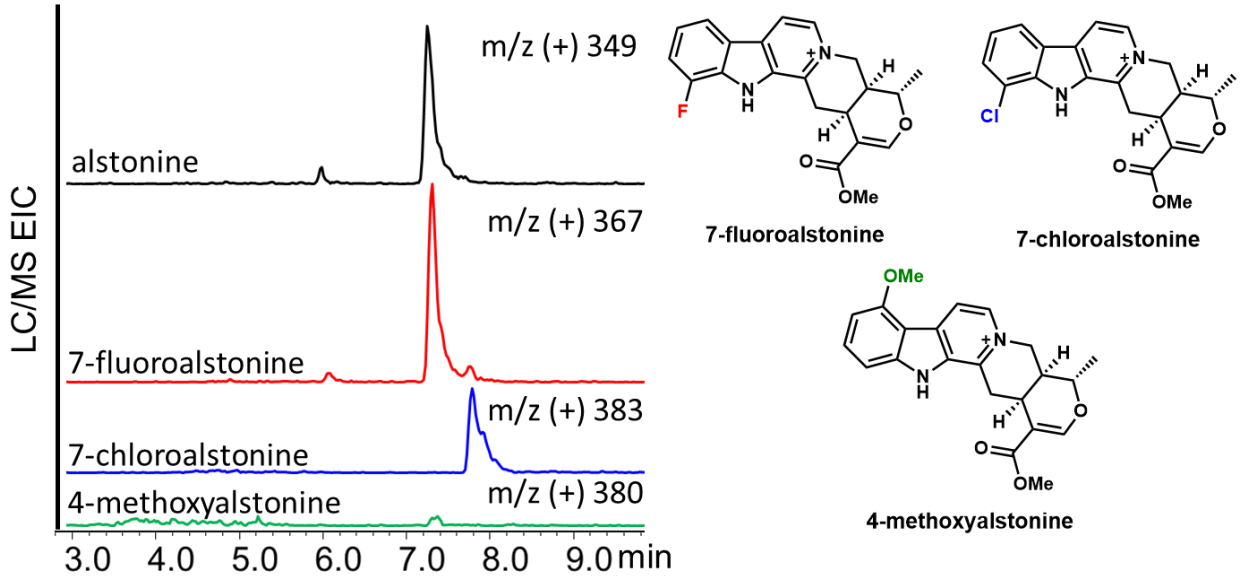


Figure 17. Production of modified alstonines.

Together these experiments support that we can leverage our MIA platform strain to access bioactive heteroyohimbine MIAs such as alstonine and novel analogs. To our knowledge, this is the first demonstrated production of tetrahydroalstonine and alstonine analogs in a microbial host.

4.3. Production of Kratom Alkaloids and Analogs

While consumption of alkaloids from kratom (*Mitragyna speciosa*) for ritual and recreation purposes has occurred for centuries, these corynanthe-type alkaloids have been subject to many studies to evaluate their therapeutic potential.[54,93] Mitragynine is one of the major accumulating alkaloids in kratom. A recent study into structural analogs of these has provided some insight into structure-activity relationships of key motifs of the corynanthean scaffold, especially about the indole ring. However, there are limitations in scope of analogs that can be accessed through traditional chemical synthesis. Following the elucidation of the first two steps in the mitragynine biosynthetic pathway by O'Connor and coworkers, we sought to investigate if our yeast platform could be used to access mitragynine pathway intermediates and novel analogs with potentially altered bioactivity.

We expressed RsSGD, MsDCS, and MsEnolMT under ADH2 promoters on a 2μ plasmid (pJM130) in yJM053 along with pVS5. Geraniol and tryptamine were fed to triplicates of the dual transformants in a standard fed-batch assay and extracted 24 hours after feeding. LC/MS analysis of culture extracts revealed the appearance of 4 new peaks, two at m/z = 355 and two m/z = 369 (Figure 18). This is the expected result as MsDCS is known to catalyze the formation of (20*R*) and (20*S*)-dihydrocorynantheine while MsEnolMT is known to catalyze the methylation of both substrates. The retention time and mass pattern of one of the m/z = 369 peaks perfectly matches a standard of (20*S*)-corynantheidine, indicating to us that we were successful in production of corynantheidine and these two enzymes from kratom are active in yeast.

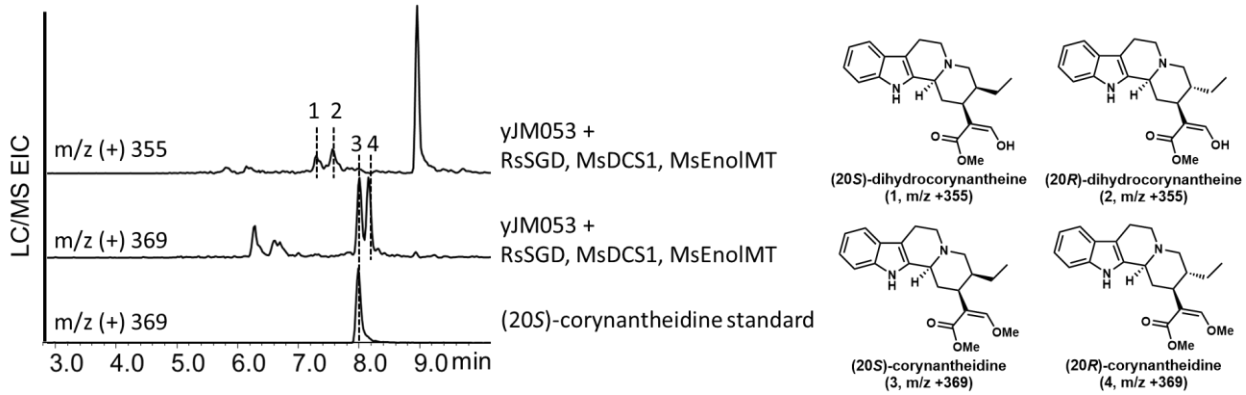
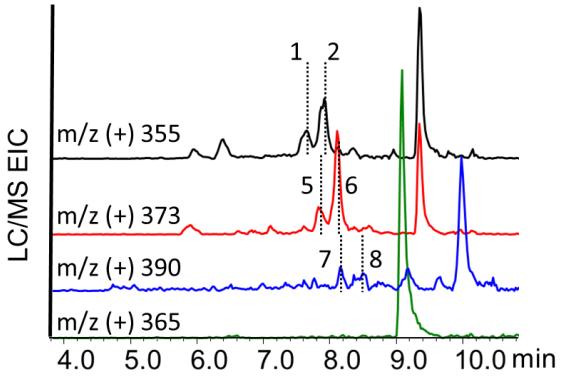
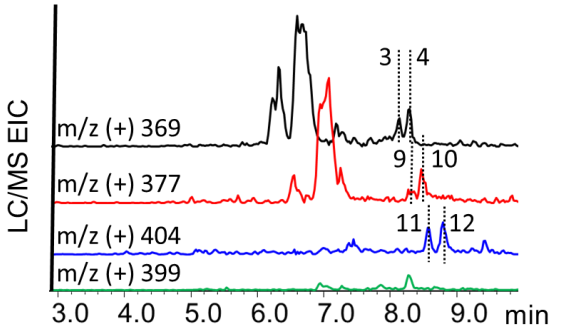


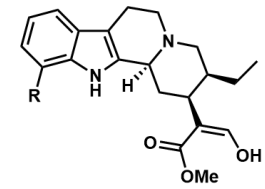
Figure 18. Production of kratom alkaloids.

The potent bioactive kratom alkaloid mitragynine is believed to form following a hydroxylation at the 4-position on the indole ring, followed by a methyl transferase on that hydroxy group to form a methoxy moiety. Since (20*S*)-corynantheidine and mitragynine only differ by this 4-methoxy group, we hypothesized we could access mitragynine by supplementing 4-methoxytryptamine to our strain. If MsDCS1 and MsEnolMT are promiscuous enough to accommodate the 4-methoxy moiety, we could circumvent the missing enzymes in the pathway to access mitragynine. In addition to feeding 4-methoxy tryptamine, we decided to follow our investigation into production of alstonine analogs through feeding 7-fluorotryptamine and 7-chlorotryptamine to our corynantheidine-producing strain as well. Biological triplicates of yJM053

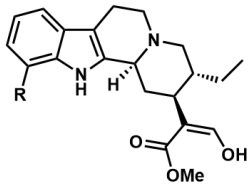
co-transformed with pVS5 and pJM130 were fed geraniol and the respective tryptamine analog in standard fed-batch procedures. Following extraction, we only observed peaks corresponding to 7-fluoro and 7-chloro analogs of the kratom alkaloids (Figure 19). There was no accumulation of 4-methoxy analogs, precluding our approach to access mitragynine without the native biosynthetic enzymes. The relative titers of each analog follows previous findings in the modified strictosidine and alstonine assays where production of the larger analogs was diminished. However, the efficient incorporation of 7-fluorotrpytamine provides a promising candidate for a novel kratom alkaloid that may have altered bioactivity.



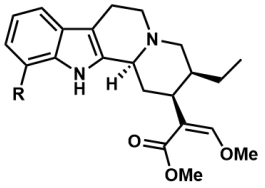




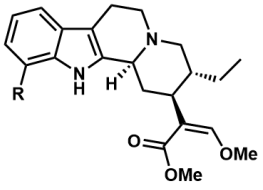
(20*S*)-dihydrocorynantheine
R = H , 1
= F, 5
= Cl, 7
= OMe



(20*R*)-dihydrocorynantheine
R = H, 2
= F, 6
= Cl, 8
= OMe



(20*S*)-corynantheidine
R = H, 3
= F, 9
= Cl, 10
= OMe



(20*R*)-corynantheidine
R = H, 4
= F, 10
= Cl, 12
= OMe

Figure 19. Production of kratom alkaloid analogs.

5. CONCLUSION

Our research herein described our efforts in the development of a yeast-based platform for production of MIAs and novel analogs. Our approach diverged from most other yeast-based platforms for natural product production by using an auto-inducible expression system that leverages diauxic shift. This approach allowed us to express over 13 heterologous enzymes with minimal growth defects to our strain. Towards production of the universal precursor strictosidine, we achieved a titer of about 60 mg/L from geraniol and tryptamine through a combination of gene expression optimization ranging from promoter selection to copy number. We demonstrated we are able to scale production and isolate strictosidine to a pure form.

While bioprospecting enzyme homologs of inefficient steps in the pathway did not result in identification of a more active variant in yeast, we affirmed that the *C. roseus* variants are optimal. Further investigations into our platform allowed us to identify that G8H integrated copy number is a key bottleneck in development of a plasmid-free strain. Still, through expression of only a single plasmid, our platform can be leveraged to access modified strictosidine analogs.

Finally, we leveraged our strictosidine platform to produce bioactive MIAs like alstonine and corynantheidine, along with novel analogs that are not easily accessible by other means and could have enhanced bioactivities. While our synthetic biology approach did not result in access to mitragynine, once the remaining biosynthetic pathway steps are revealed, our platform can be quickly adapted to accommodate those enzymes. We also identified that SGD activity is the next key bottleneck that must be addressed for optimal titers. Approaches to evolve SGD towards a superior variant can utilize production of a molecular probe like the fluorescent alstonine for high-throughput screening.

6. MATERIALS AND METHODS

6.1. Plasmid and Strain Construction

All yeast expression plasmids were cloned using yeast homologous recombination. Fragments for recombination were amplified using Q5 polymerase (NEB) with ~35 bp of homology overlap to subsequent fragments and column purified using a Zymoclean Gel DNA Recovery Kit (Zymo Research). Strictosidine pathway genes from C. roseus and putative 7DLHs were codon-optimized and synthesized by Gen9 or IDT (Appendix B). The auto-inducible ADH2 and ADH2-like promoters and high-capacity terminators were amplified from S. cerevisiae genomic DNA. Amplified fragments for cloning were transformed into yeast using the standard lithium acetate method,[94] plated onto the corresponding supplemental complete media (SC) deficient for uracil, leucine, and/or histidine. After 48 hours of outgrowth, the plasmid was extracted from clumps of colonies using a Zymoprep Yeast Plasmid Miniprep I kit (Zymo Research). The yeast miniprep solution was then transformed into electrocompetent TOP10 Escherichia coli cells for plasmid propagation using electroporation and plated onto LB agar supplemented with 100 mg/L carbenicillin. Several colonies after 16 hours of outgrowth were inoculated into liquid media supplemented with carbenicillin, grown overnight, and miniprepped using a Zyppy Plasmid Miniprep Kit (Zymo Research). Successful plasmid constructs were identified through restriction digest (NEB) and then verified by Sanger sequencing (Laragen). Genomic integration of expression cassettes was achieved through a two-stage strategy. A LEU2 marker was first integrated at the genomic loci of choice using a linearized donor DNA from a plasmid containing 300–500 bp of homology flanking the LEU2 marker, following the standard transformation protocol as described above. Next, the linearized expression cassette of choice with 300–500 bp of homology from a homology donor plasmid was co-transformed with a plasmid containing a CRISPR-Cas9 system[95] encoding an sgRNA targeting the LEU2 marker. The transformed yeast was then inoculated into 3 mL of YPD media for outgrowth for 14 hours and

then 200 µL was plated onto YPD agar plates supplemented with 400 mg/L G418 sulfate. After 48 hours of growth, colonies were first screened by counter selection on SC agar plates deficient for leucine and then by colony PCR. Successful integrations were subject to further characterization and verification by genomic DNA extraction using a YeaStar Genomic DNA Kit (Zymo Research) and subsequent PCR and Sanger sequencing.

6.2. Culture and Fed-Batch Assay Conditions

For all plasmid-based yeast assays, single colonies were picked and inoculated into 500 µL of the respective SC media deficient of uracil, leucine, and/or histidine and grown overnight in a Lab-Therm LX-T (Adolf Kuhner) incubator shaker at 280 RPM and 28 °C. This seed culture was then inoculated into 500 µL YPD in a 96 deep-well plate or 1.5 mL YPD in culture tube to an OD600 of 0.1. In plasmid-free based strain assays, single colonies were directly inoculated in 1.5 mL of YPD. 96 deep-well plate cultures are covered with AeraSeal film (Excel Scientific) and grown at 28 °C, shaking at 400 RPM. All 1.5 mL YPD cultures are grown at 28 °C and shaken at 280 RPM. After 24 hours of outgrowth in rich media, strains were fed geraniol or nepetalactol and tryptamine from 200 mM stocks dissolved in ethanol to a culture concentration of 2 mM.

6.3. Protein Purification

The genes encoding the protein of interest were cloned into a pET-28a vector via HiFi DNA assembly (New England Biolabs). These vectors were individually transformed into SolBL21 electrocompetent *E. coli* cells. Single colonies of these transformations were inoculated into 10 mL of LB media supplemented with 50 mg/L kanamycin and grown overnight. These overnight cultures were used to inoculate 1 liter LB cultures supplemented with 50 mg/L kanamycin which were grown at 37 °C until an OD_{600} of ~0.6. Then, the cultures were supplemented with IPTG to a concentration of 100 uM and protein expression was induced at 16 °C for 16 hours. Following induction, the cell pellet was isolated via centrifugation, mixed with 30 mL of A10 buffer (50 mM

sodium phosphate, 500 mM sodium chloride, 10% glycerol, 10 mM imidazole, pH= 8) and lysed on ice via sonication. The soluble lysate was separated from the insoluble fraction via centrifugation and mixed with 1 mL of HisPur Ni-NTA resin (Thermo Scientific) and slowly mixed at 4 °C for 2 hours. This mixture was loaded into a protein purification column (6 mL capacity) and washed with five column volumes of A10 buffer, A25 (same as A10, but with 25 mM imidazole), A50, and A100 buffer sequentially. Protein was eluted with five column volumes of A250 buffer. Fractions from each wash were collected and verified for protein content on an SDS-page gel. Fractions containing protein of interest were pooled and concentrated using Amicon concentrators (MilliporeSigma), aliquoted and flash-frozen with liquid nitrogen.

6.4. In-vitro Reactions

In vitro reactions were prepared in sodium phosphate buffer at pH = 8. Depending on the experiment, 50 nM of purified SGD, and/or 100 uM strictosidine, were added to each 100 µL reaction. Reactions were incubated at 30 °C for 1-2 hours, with additional protein and substrates being added as necessary for the experiment. Reactions were halted by the addition of 100 µL of methanol. Following centrifugation, the buffer/methanol supernatant was analyzed on LC/MS to monitor substrate and production levels (see Section 6.6).

6.5 Growth Assays

All strains were grown overnight in biological triplicate in 1 mL YPD or respective selective media. These overnight cultures were used to inoculate 100 µL of YPD to a starting OD600 of 0.01 in a 96-well clear plate. The plate was then sealed and placed into an Infinite M200 plate reader (TECAN) for incubation. Cultures were continuously shaken at 280 RPM at 28 °C with OD600 measurements taken every 15 min for 24 hours.

6.6. Monoterpene Indole Alkaloid Intermediate Extraction and Analysis

Samples were extracted 24 hours after feeding substrates. 200 µL of whole culture was extracted with 200 µL acetone and vortexed for 30 s. The samples were then centrifuged for 10 min at maximum speed. The supernatant is then removed and placed into a clean tube, and an equal volume of MilliQ water is added to dilute the sample. For MIA analysis downstream strictosidine, samples were extracted with 200 µL 3:1 ethyl acetate-acetone mixture and vortexed for 30 seconds. Following centrifugation for 5 minutes at maximum speed, the organic top layer was transferred to a clean tube. The organic layer was evaporated using a vacuum concentrator and resuspended in 100 µL methanol. All samples were then analyzed on a Shimadzu 2020 EV LC/MS equipped with a Phenomenex Kinetex C18, 1.7 µm, 100 Å, 2.1 × 100 mm reverse-phase column. Both positive- and negative-mode electrospray ionization were performed with a linear gradient of 5–95% acetonitrile-H2O spiked with 0.1% formic acid over 15 min and then 95% acetonitrile for 3 min with a flow rate of 0.3 mL/min. High-resolution MS/MS data was collected on an Agilent 6545 LC/Q-TOF MS with a 25 V collision voltage. Strictosidine and pathway intermediate peaks were verified by comparison to available standards and quantified using calibration curves generated from standards. 7-Deoxyloganic acid was quantified using loganic acid as a proxy for LC/MS mass response because sufficient quantities of the standard were not able to be obtained. Loganic acid and loganin standards were purchased from ChemFaces. Strictosidine standard was a gift from Neil Garg's lab, UCLA. (20*S*)-corynantheidine, (20*S*)-9-hydroxycorynantheidine and mitragynine standards were a gift from Christopher McCurdy's lab, University of Florida.

6.7. Strictosidine Purification

Yeast strain yJM025 co-transformed with pJB204 and pJM057 used for the production of strictosidine at a 1 L scale. Following outgrowth, geraniol and tryptamine in ethanol were added to a concentration of 2 mM each. 24 hours after feeding, the culture was centrifuged to separate the cell pellet and culture supernatant. The supernatant was subjected to HP-20 column chromatography (water to MeOH). The MeOH eluate fraction was applied to a Sephadex LH-20 column (MeOH) to give three fractions (frs. 1–3). Fr. 2 was subjected to ODS MPLC and carried out on a RediSep Gold Reverse-phase C18 column (TELEDYNE, Lincoln, USA), (MeOH/H2O, 0:100→100:0) to give six fractions (frs. 2.1–2.6), and then fr. 2.5 was further separated by Sephadex LH-20 column chromatography (CHCl3/MeOH, 5:5) to obtain three fractions (frs. 2.5.1–2.5.3). Fr. 2.5.2 was purified by ODS HPLC on a COSMOSIL 5C18-AR-II column(φ10 × 250 mm, MeCN/H2O/formic acid, 20:80:0.1) to furnish strictosidine. The 1D NMR spectrum was obtained on a Bruker AV500 spectrometer for structure verifications and compared with a standard from Neil Garg's lab, UCLA. The resonances of residual methanol (δH 3.30 and δC 49.0) were used as internal references for the 1H and 13C NMR spectra. High-resolution MS/MS data were collected on an Agilent 6545 LC/Q-TOF MS with a collision voltage of 25 V.

Supplementary Figures

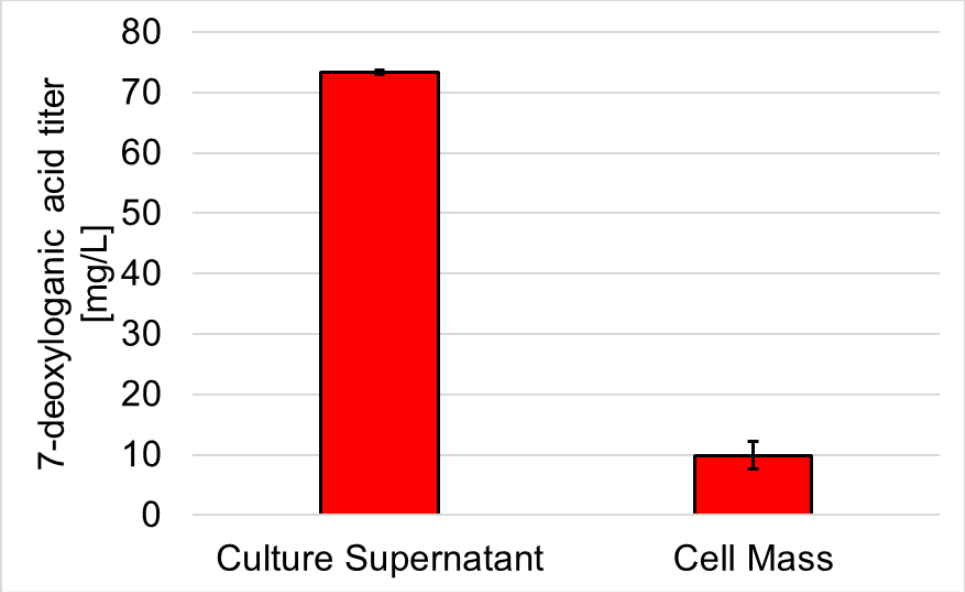


Figure S1. Distribution of 7-Deoxyloganic Acid in Yeast Culture. Titers of 7-deoxyloganic acid extracted from culture supernatant and cell mass separately. Bars indicate the mean of biological duplicates with the error bars representing the standard error.

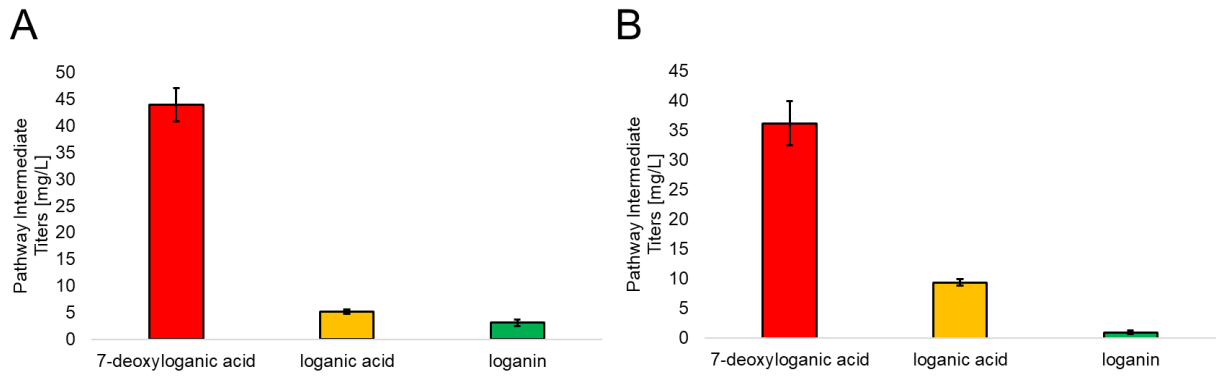


Figure S2. Effects of Varied P450 Copy Number on Pathway Intermediate Accumulation. (**A**) Titers of pathway intermediates from yJM010 co-transformed with high-copy pJB152 and pJB040. (**B**) Titers of pathway intermediates from yJM025 transformed with low-copy pJM057. Bars indicate the mean of biological triplicates with the error bars representing the standard error.

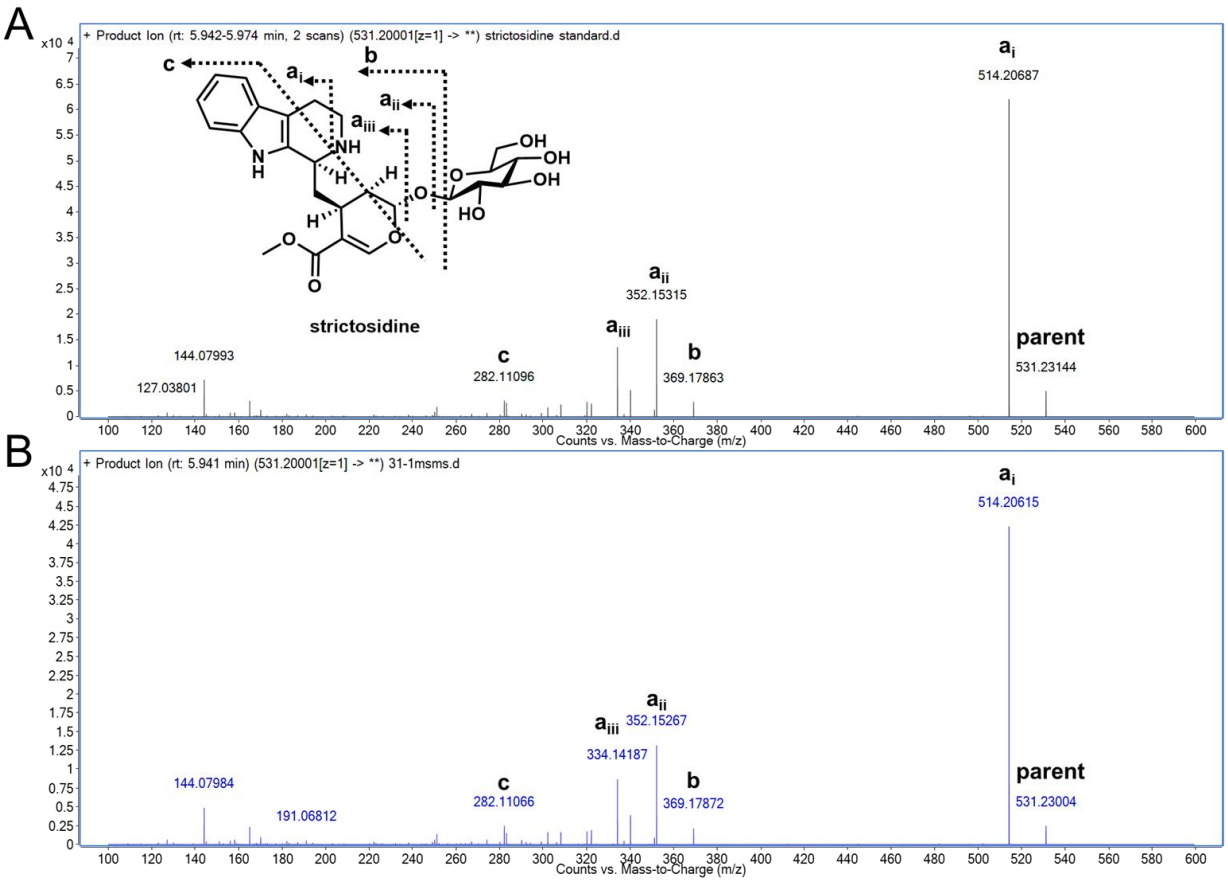


Figure S3. Strictosidine MS/MS Spectra. (**A**) MS/MS fragmentation pattern of strictosidine standard with predominant fragments. (**B**) MS/MS fragmentation pattern of strictosidine from yeast culture.

Figure S4. ^{1}H-^{1}H COSY spectrum of strictosidine in CD_3OD (500 MHz).

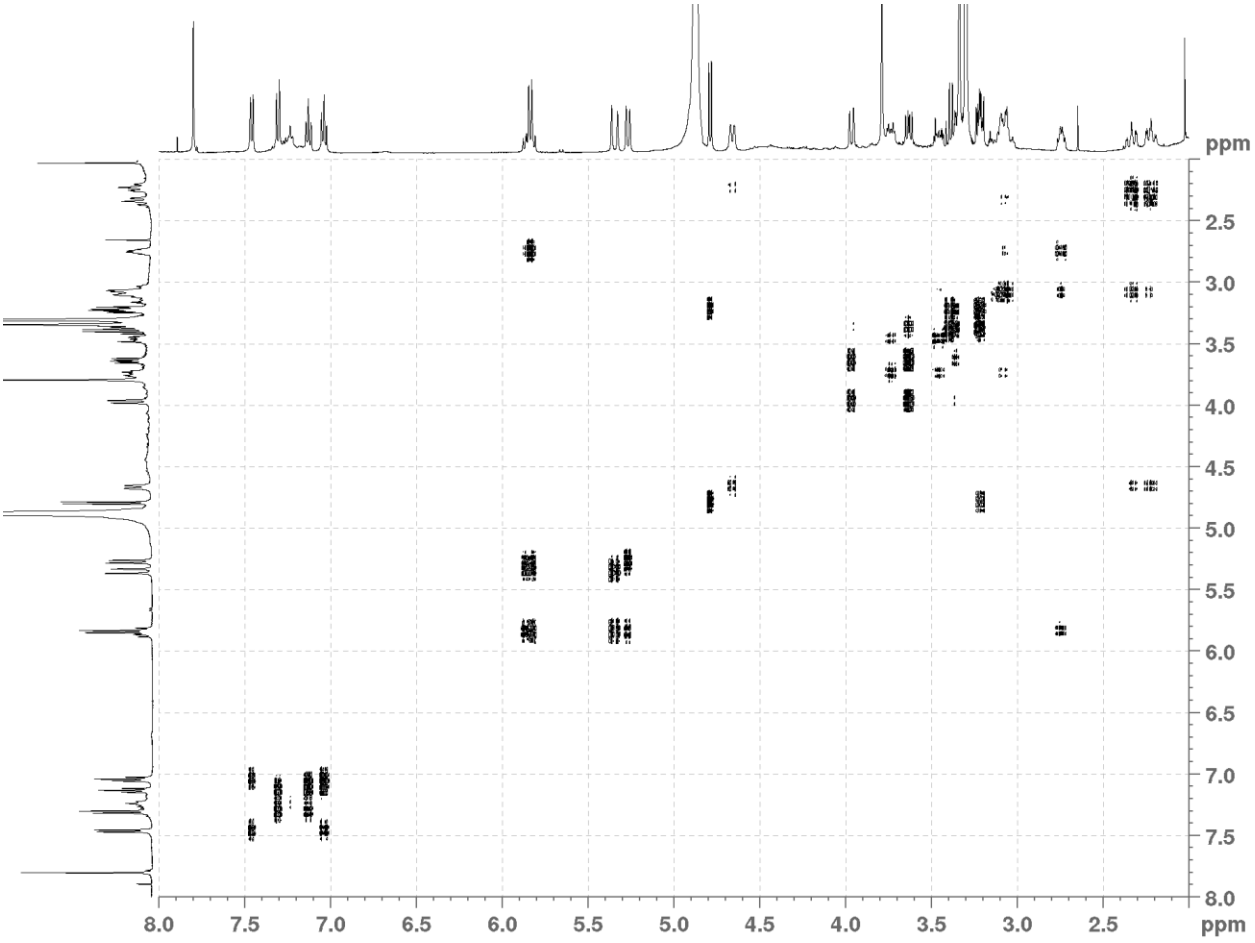


Figure S5. HSQC spectrum of strictosidine in CD_3OD (500 MHz).

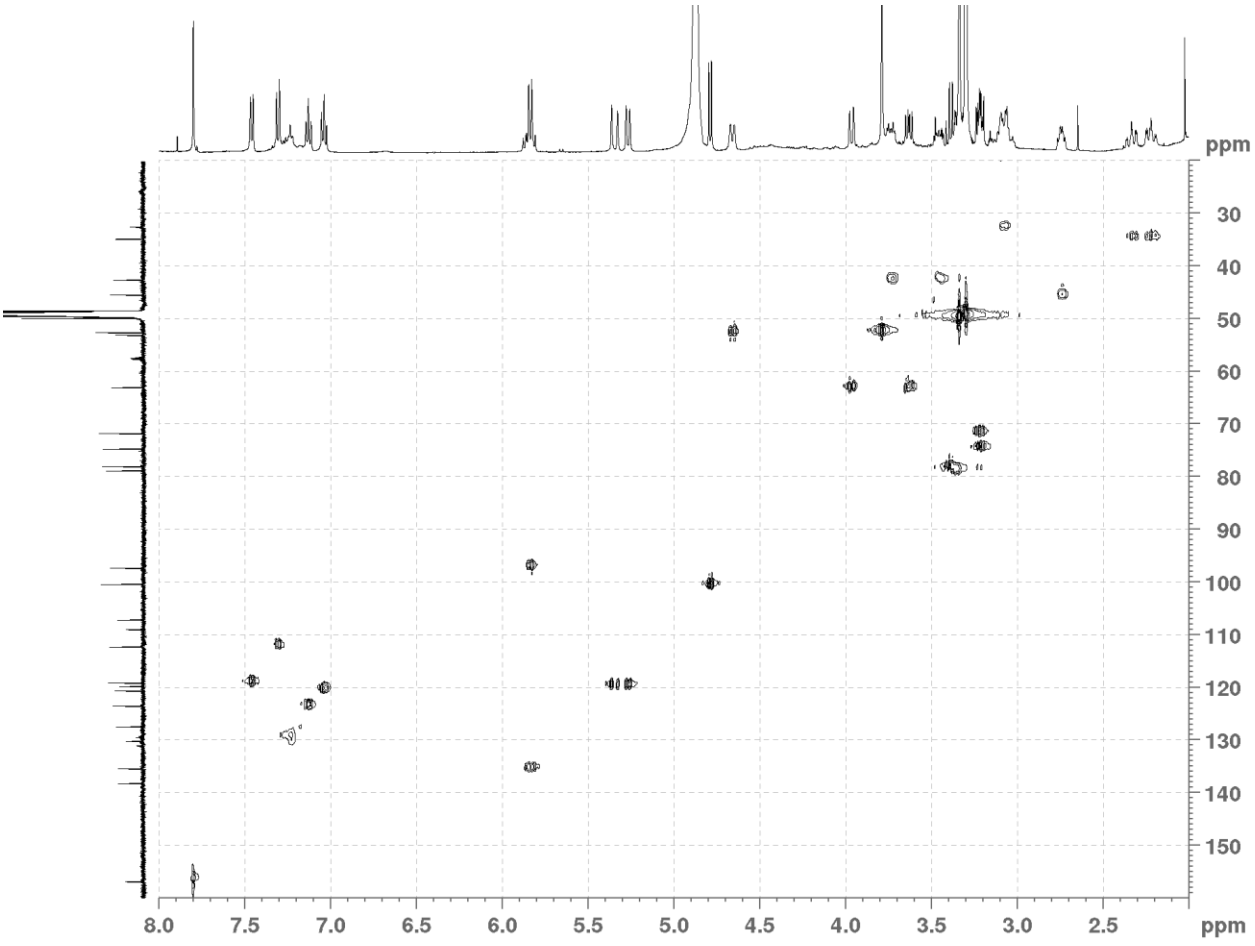


Figure S6. HMBC spectrum of strictosidine in CD_3OD (500 MHz).

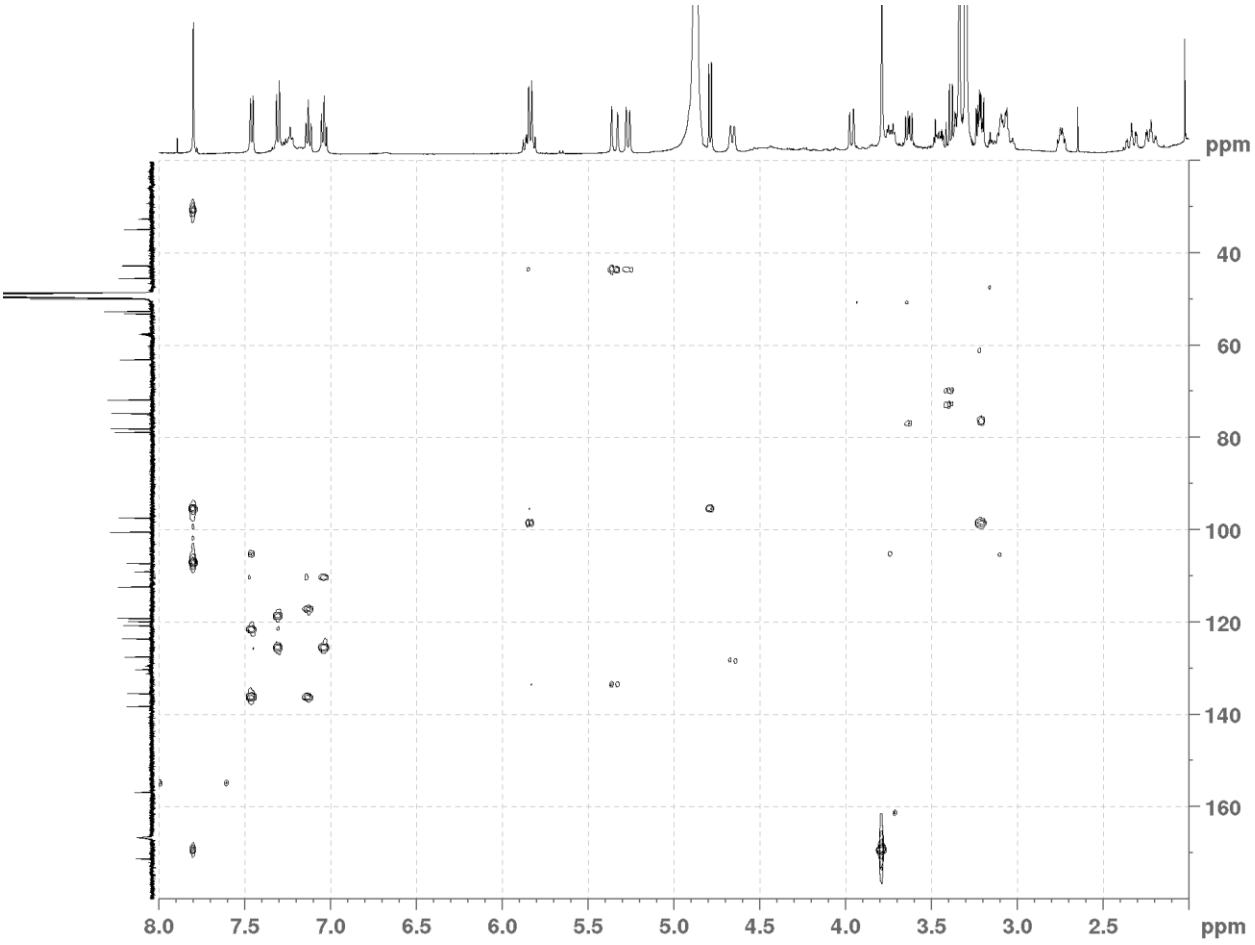


Figure S7. NOESY spectrum of strictosidine in CD_3OD (500 MHz, H-3/H-15 interaction highlighted).

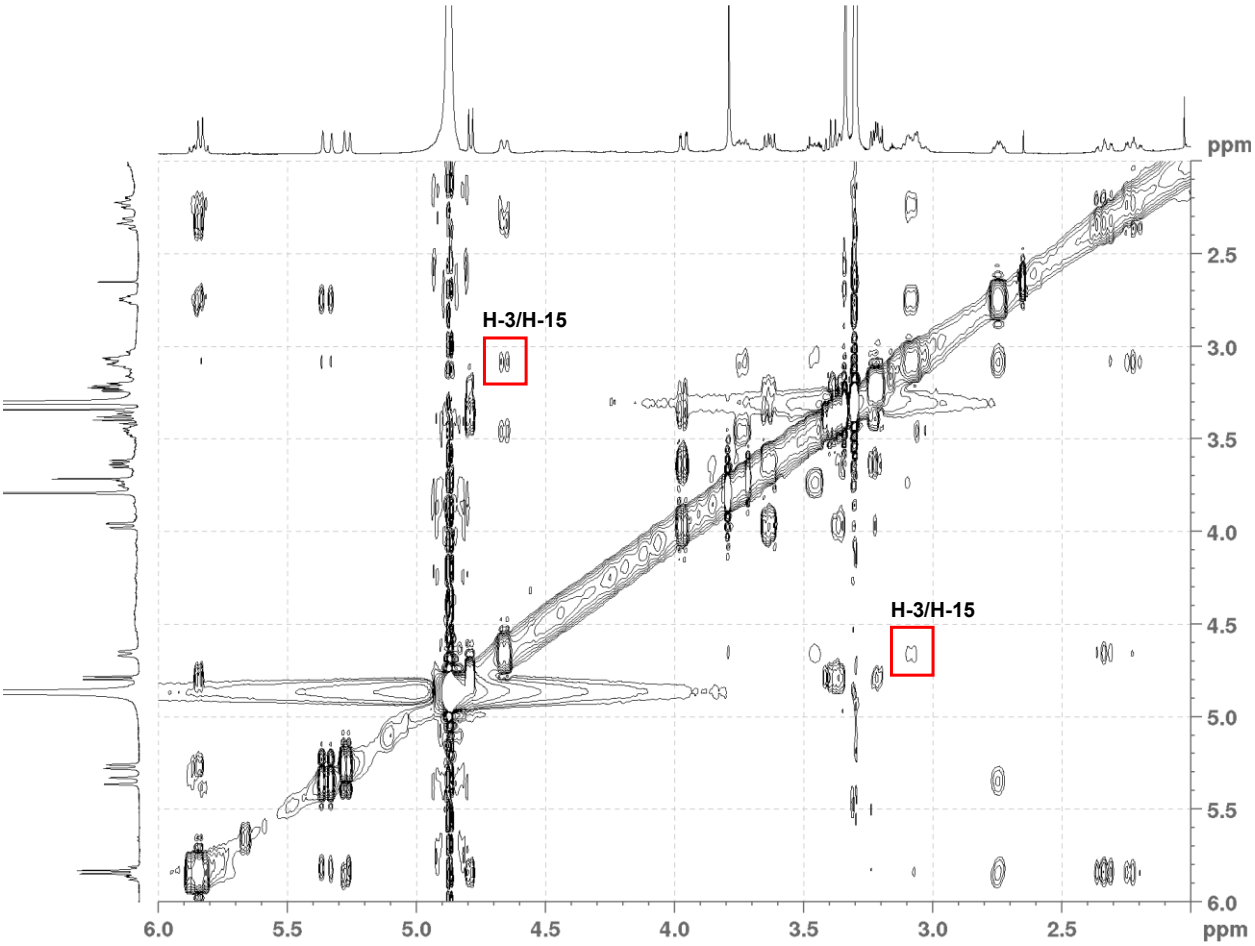
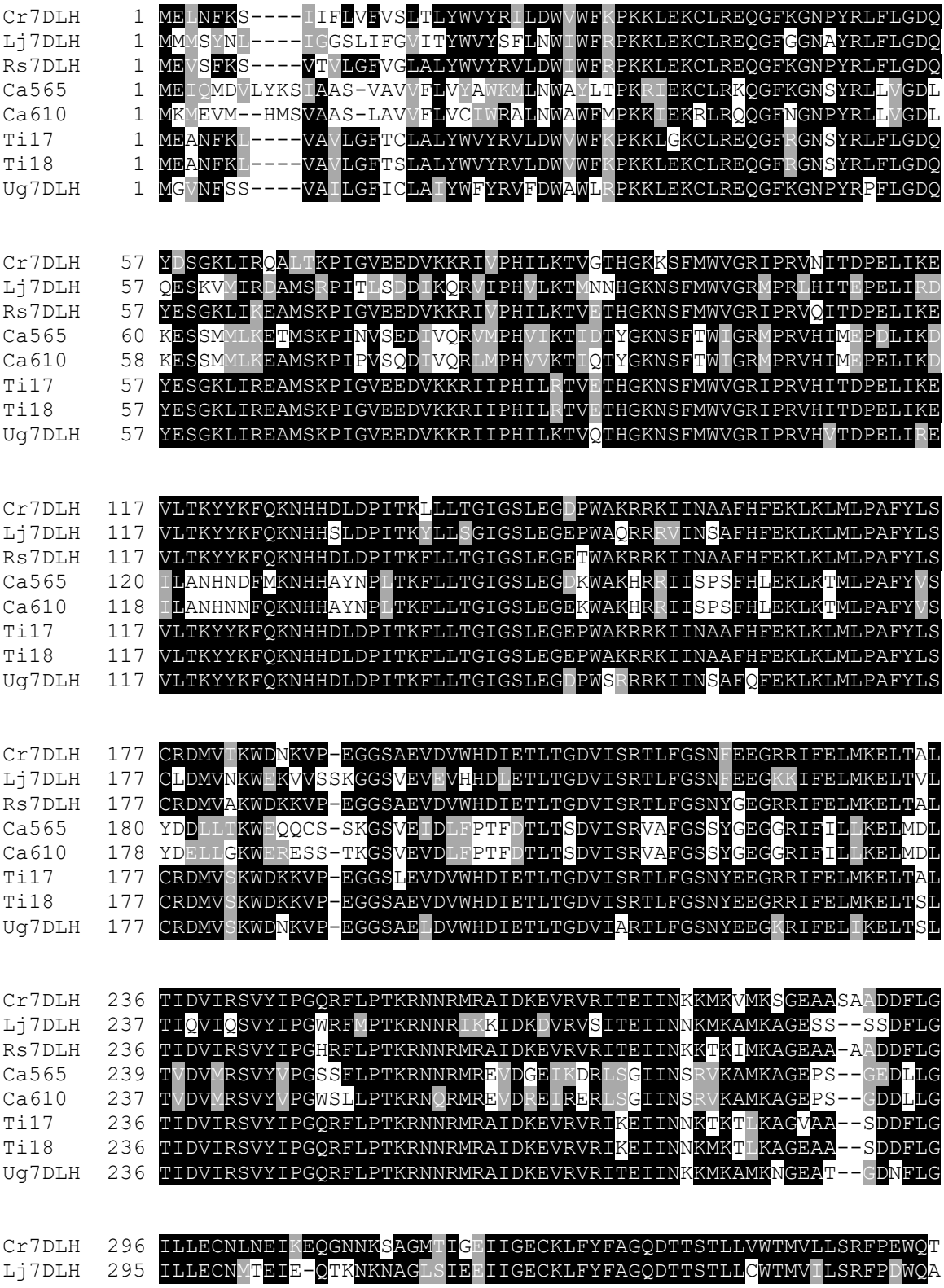


Figure S8. Sequence Alignment of 7DLH Enzymes.



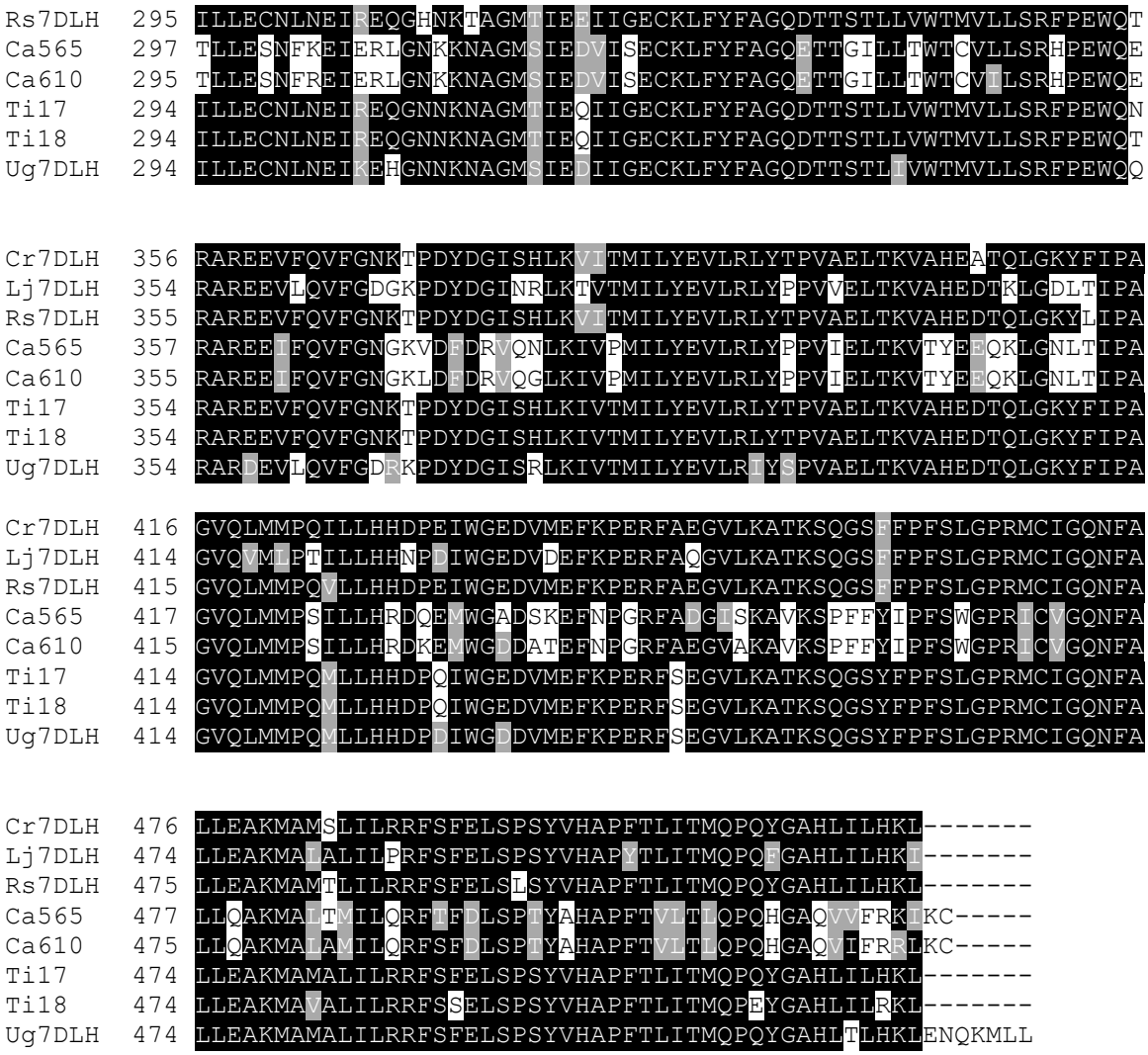
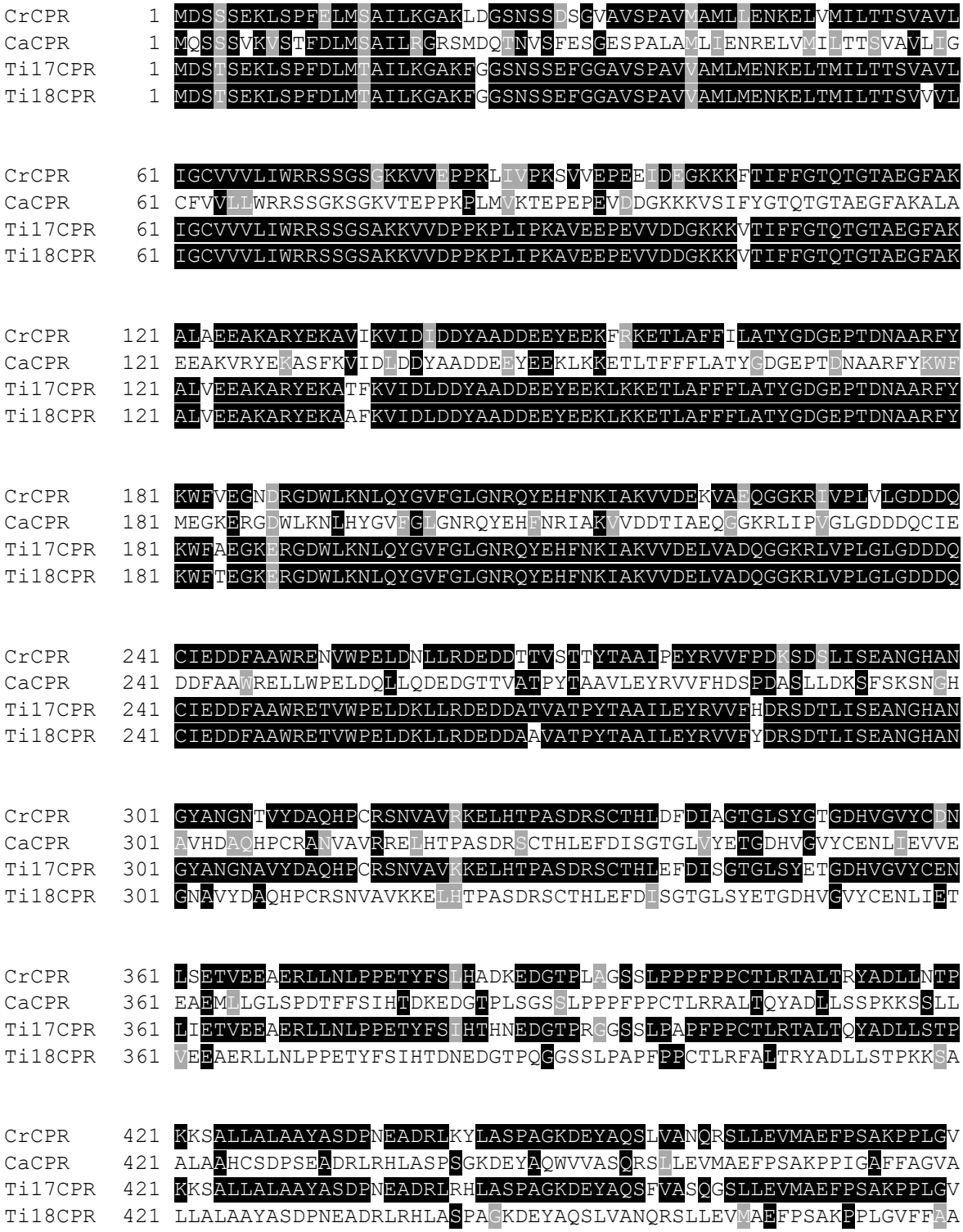

Rs7DLH 295 ILLECNLNEIREQGHNKTAGMTIEEIIGECKLFYFAGQDTTSTLLVWTMVLLSRFPEWQT
Ca565 297 TLLESNFKEIERLGNKKNAGMSIEDVISECKLFYFAGQETTGILLTWTCVLLSRHPEWQE
Ca610 295 TLLESNFREIERLGNKKNAGMSIEDVISECKLFYFAGQETTGILLTWTCVILSRHPEWQE
Ti17 294 ILLECNLNEIREQGNNKNAGMIIEQIIGECKLFYFAGQDTTSTLLVWTMVLLSRFPEWQN
Ti18 294 ILLECNLNEIREQGNNKNAGMTIEQIIGECKLFYFAGQDTTSTLLVWTMVLLSRFPEWQT
Ug7DLH 294 ILLECNLNEIKEHGNNKNAGMSIEDIIGECKLFYFAGQDTTSTLIVWTMVLLSRFPEWQQ
Cr7DLH 356 RAREEVFQVFGNKTPDYDGISHLKVITMILYEVLRLYTPVAELTKVAHEATQLGKYFIPA
Lj7DLH 354 RAREEVLQVFGDGKPDYDGINRLKTVTMILYEVLRLYPPVVELTKVAHEDTKLGDLTIPA
Rs7DLH 355 RAREEVFQVFGNKTPDYDGISHLKVITMILYEVLRLYTPVAELTKVAHEDTQLGKYLIPA
Ca565 357 RAREEIFQVFGNGKVDFDRVQNLKIVPMILYEVLRLYPPVIELTKVTYEEQKLGNLTIPA
Ca610 355 RAREEIFQVFGNGKLDFDRVQGLKIVPMILYEVLRLYPPVIELTKVTYEEQKLGNLTIPA
Ti17 354 RAREEVFQVFGNKTPDYDGISHLKIVTMILYEVLRLYTPVAELTKVAHEDTQLGKYFIPA
Ti18 354 RAREEVFQVFGNKTPDYDGISHLKIVTMILYEVLRLYTPVAELTKVAHEDTQLGKYFIPA
Ug7DLH 354 RARDEVLQVFGDRKPDYDGISRLKIVTMILYEVLRIYSPVAELTKVAHEDTQLGKYFIPA
Cr7DLH 416 GVQLMMPQILLHHDPEIWGEDVMEFKPERFAEGVLKATKSQGSFFPFSLGPRMCIGQNFA
Lj7DLH 414 GVQVMIPTILLHHNPDIWGEDVDEFKPERFAQGVLKATKSQGSFFPFSLGPRMCIGQNFA
Rs7DLH 415 GVQLMMPQVLLHHDPEIWGEDVMEFKPERFAEGVLKATKSQGSFFPFSLGPRMCIGQNFA
Ca565 417 GVQLMMPSILLHRDQEMWGADSKEFNPGRFADGISKAVKSPFFYIPFSWGPRICVGQNFA
Ca610 415 GVQLMMPSILLHRDKEMWGDDATEFNPGRFAEGVAKAVKSPFFYIPFSWGPRICVGQNFA
Ti17 414 GVQLMMPQMLLHHDPQIWGEDVMEFKPERFSEGVLKATKSQGSYFPFSLGPRMCIGQNFA
Ti18 414 GVQLMMPQMLLHHDPQIWGEDVMEFKPERFSEGVLKATKSQGSYFPFSLGPRMCIGQNFA
Ug7DLH 414 GVQLMMPQMLLHHDPDIWGDDVMEFKPERFSEGVLKATKSQGSYFPFSLGPRMCIGQNFA
Cr7DLH 476 LLEAKMAMSLILRRFSFELSPSYVHAPFTLITMQPQYGAHLILHKL-------
Lj7DLH 474 LLEAKMALALILPRFSFELSPSYVHAPYTLITMQPQFGAHLILHKI-------
Rs7DLH 475 LLEAKMAMTLILRRFSFELSLSYVHAPFTLITMQPQYGAHLILHKL-------
Ca565 477 LLQAKMALTMILQRFTFDLSPTYAHAPFTVLTLQPQHGAQVVFRKIKC-----
Ca610 475 LLQAKMALAMILQRFSFDLSPTYAHAPFTVLTLQPQHGAQVIFRRLKC-----
Ti17 474 LLEAKMAMALILRRFSFELSPSYVHAPFTLITMQPQYGAHLILHKL-------
Ti18 474 LLEAKMAVALILRRFSSELSPSYVHAPFTLITMQPEYGAHLILRKL-------
Ug7DLH 474 LLEAKMAMALILRRFSFELSPSYVHAPFTLITMQPQYGAHLTLHKLENQKMLL

Figure S9. Sequence Alignment of CPR Enzymes.



```
CrCPR    481 FFAAIAPRLQPRFYSISSSPRMAPSRIHVTCALVYEKTPGGRIHKGVCSTWMKNAIPLEE
CaCPR    481 PRLQPRYYSISSSPRMAPSRIHVTCALVFEKTPVGRIHKGVCSTWMKNAVPLDESRDCSW
Ti17CPR  481 FFAAIAPRLQPRFYSISSSPRMAPSRIHVTCALVYEKTPGGRIHKGVCSTWMKNAIPLEE
Ti18CPR  481 IAPRLQPRFYSISSSPRMAPSRIHVTCALVYEKTPGGRIHKGVCSTWMKNAIALEESRDC

CrCPR    541 SRDCSWAPIFVRQSNFKLPADPKVPVIMIGPGTGLAPFRGFLQERLALKEEGAELGTAVF
CaCPR    541 APIFVRQSNFKLPADTKVPVLMIGPGTGLAPFRGFLQERLALKEAGAELGPAILFFGCRN
Ti17CPR  541 SRDCSWAPIFVRQSSFKLPADPKVPIIMIGPGTGLAPFRGFLQERLALKEGGAELGPAIF
Ti18CPR  541 SWAPIFIRQSNFKLPADPKVPIIMIGPGTGLAPFRGFLQERLALKEEGAELGPAIFFFGC

CrCPR    601 FFGCRNRKMDYIYEDELNHFLEIGALSELLVAFSREGPTKQYVQHKMAEKASDIWRMISD
CaCPR    601 RQMDYIYEDELNNFVETGALSELIVAFSREGPKKEYVQHKMMEKASDIWNMISQEGYIYV
Ti17CPR  601 FFGCRNSKMDYIYENELNHFLETGALSELDLAFSREGPTKQYVQHKMAAKASDIWRMISD
Ti18CPR  601 RNSKMDYIYENELNHFVETGALSELDLAFSREGPTKQYVQHKMVAKASDIWRMISDGAYV

CrCPR    661 GAYVYVCGDAKGMARDVHRTLHTIAQEQGSMDSTQAEGFVKNLQMTGRYLRDVW
CaCPR    661 CGDAKGMARDVHRTLHTIVQEQGSLDSSKTESMVKNLQMNGRYLRDVW------
Ti17CPR  661 GAYVYVCGDAKGMARDVHRTLHTIAQEQGSMHSSKSESFVKNLQISGRYLRDVW
Ti18CPR  661 YVCGDAKGMARDVHRTLHTIAQEQGSMDSSKSESFVKNLQISGRYLRDVW----
```

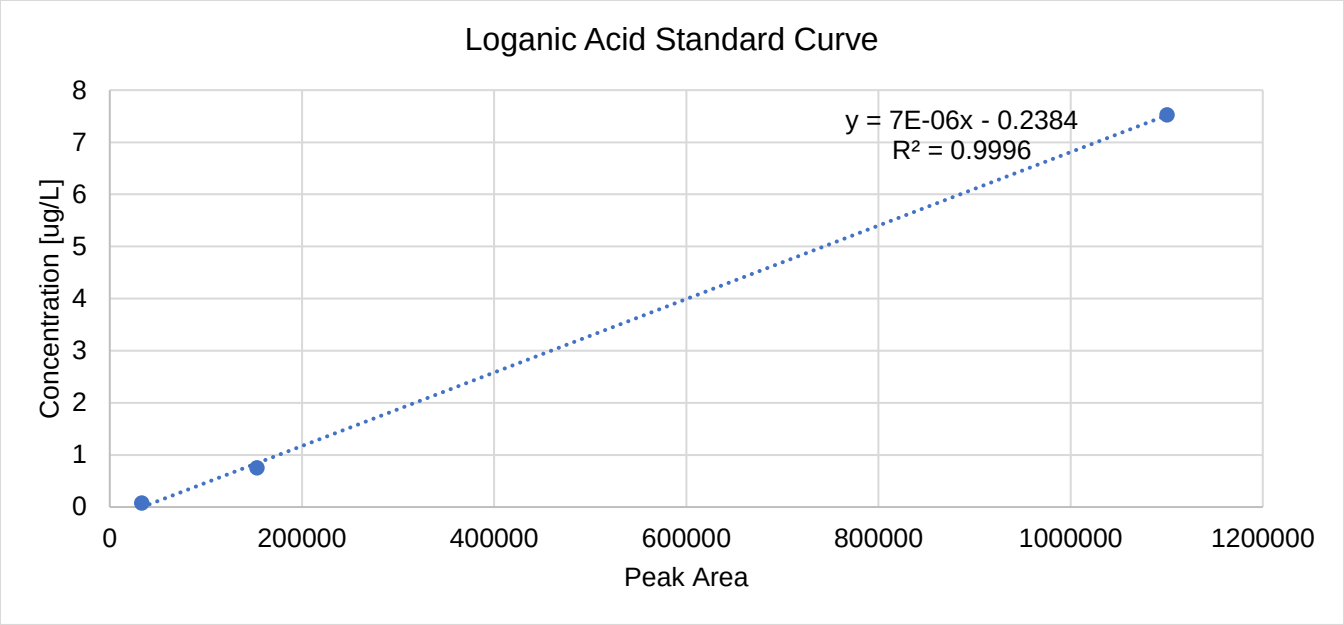


Figure S10. Loganic Acid Standard Curve. Different concentrations of loganic acid standard were measured on LC/MS where the area under the peak was recorded and plotted against concentration.

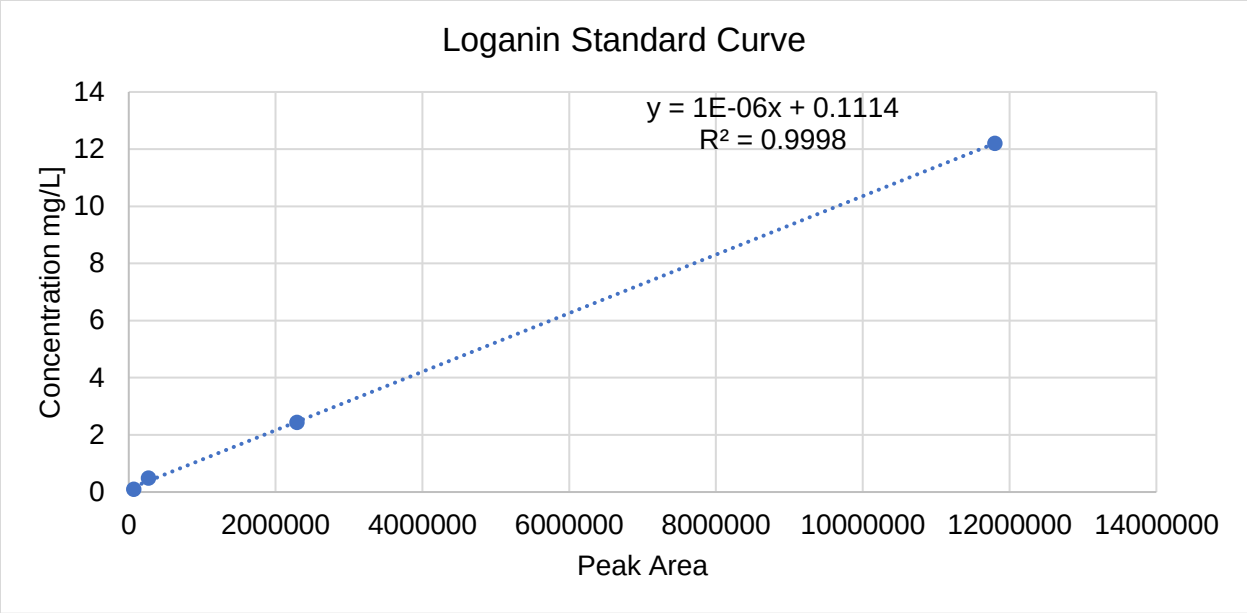


Figure S11. Loganin Acid Standard Curve. Different concentrations of loganin standard were measured on LC/MS where the area under the peak was recorded and plotted against concentration.

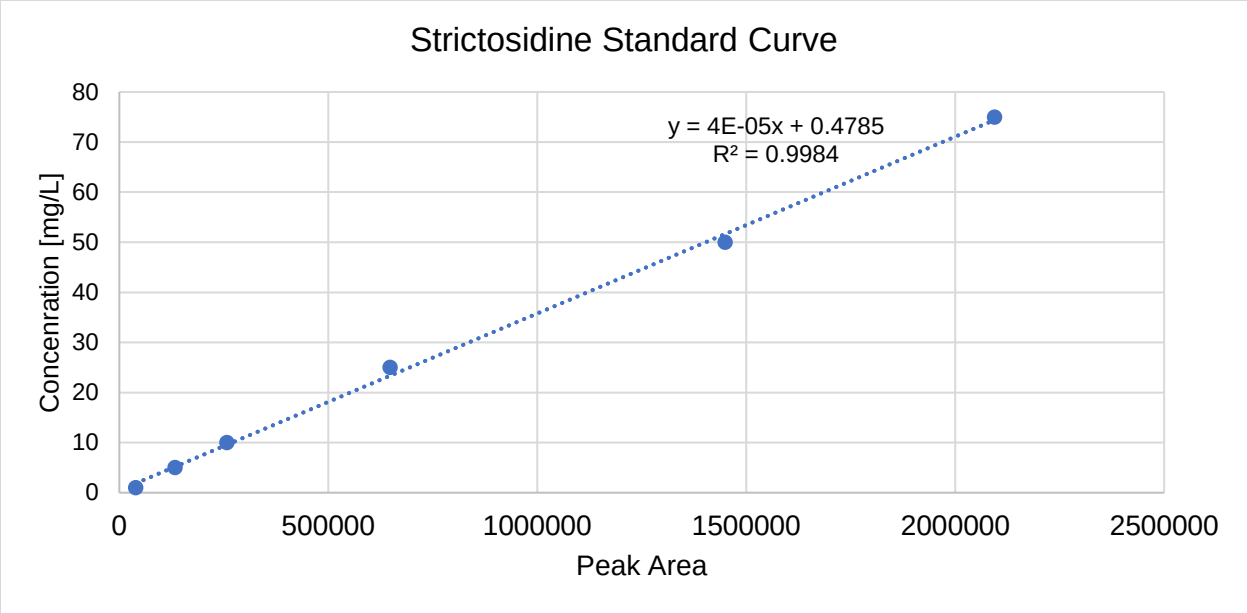


Figure S12. Strictosidine Standard Curve. Different concentrations of strictosidine standard from the Neil Garg lab at UCLA[96] were measured on LC/MS where the area under the peak was recorded and plotted against concentration.

7. APPENDICES

Appendix A – Primers Used in this Study

Primer Name	Sequence
1-1 Misa Ura3-Up-F	TGAAACTAGGGAAGACAAGCAACG
1-2 Misa Ura3-Down-R	GTTCTTGGAAACGCTGCCC
1-3 Misa Ty12-Up-F	ACATTGAACGGAAGTGCCGC
1-4 Misa Ty12-Down-R	CTCTCAGAACGCAAAGCGG
1-5 Misa Ho-Up-F	AATTGTACTACCGCTGGGCG
1-6 Misa Ho-Down-R	GAAGAGAGTTGTCACCAAGGCC
1-7 Misa OYE3up-ADH2p F	CCAAATCACGGATGTGGAAAACTGATCACGTGCTTCGCAAAACGTAGGGGCAAACAAACG
1-8 Misa SPG5t-OYE3down R	TCCCTTTGAACAGCGCGCGGGCACGAGAAAGCGCTTATTTTCTGCCGAATTTTCATGAAG
1-9 Misa OYE3up-homology F	GGAGCTTATTCCCGCACGCTCACATGGTAATTTGCGCCAAATCACGGATGTGGAAAACTG
1-10 Misa OYE3down-homology R	TACGTCAATGGGCTTGCAAGCATAAAAAAGTCATTTTATTATTCCCTTTGAACAGCGCGC
1-11 Misa AHD7-ADH2p F	GCTGTAGATCAGGGACTATGCGAGCGACAAGTCAGAGCAAAACGTAGGGGCAAACAAACG
1-12 Misa SPG5t-ADH7 R	TAAAACTTACTGCTCTGCACTGTTGTCGAGAGGCTTATTTTCTGCCGAATTTTCATGAAG
1-13 Misa AHD7up-homology F	CCGGAGTTGTTTACACACATGTCTCTTTTTGGATTAATGCTGTAGATCAGGGACTATGCG
1-14 Misa AHD7down-homology R	TGGGTAAAACCCTGCACACATTTCGTATTGAATAAAACTTACTGCTCTGCACTGTTGTCG
1-15 Misa pJBdUra3 4	ATGATCACCATCAAAGAAGGTTAATGGTTTTAGAGCTAGAAATAGCAAGTTAAAATAAGG
1-16 Misa pJBdUra3	TTTCTAGCTCTAAAACCATTAACCTTCTTTGATGGTGATCATTTATCTTTCACTGCGGAG
1-17 Misa pJBdTy12 4	ATGATCGTATTTGATGAATAATTTGTGTTTTAGAGCTAGAAATAGCAAGTTAAAATAAGG
1-18 Misa pJBdTy12 2	TTTCTAGCTCTAAAACACAAATTATTCATCAAATACGATCATTTATCTTTCACTGCGGAG
1-19 Misa pJBdHO 4	ATGATCGACATTTATGACGCGGGCAGGTTTTAGAGCTAGAAATAGCAAGTTAAAATAAGG
1-20 Misa pJBdHO 2	TTTCTAGCTCTAAAACCTGCCCGCGTCATAAATGTCGATCATTTATCTTTCACTGCGGAG
1-21 jm ori-dURA3 F	TCAGGGGGGCGGAGCCTATGGAAAAACGCCGGAGACTATTTTCATTGACCGAATCAGAG
1-22 jm dURA3 Up-LEU2 R	CTGAAACCACAGCCACATTAACC
1-23 jm LEU2-dURA3 Down F	GCTTCATGGCCTTTATAAAAAGGAACTATCC
1-24 jm dURA3 Down-2uori R	TGTTCTACAAAATGAAGCACAGATGCTTCGTTGTTTCGATTGTTTTACGTTTGAGGC
1-25 jm ori-dTY12 F	TCAGGGGGGCGGAGCCTATGGAAAAACGCCGATCTATTAGCTGAACACGGTATCGC

1-26 jm dTY12 Up-LEU2 R	AATTATTCATCAAATACATCTCGATATCCATATTTTGG
1-27 jm LEU2-dTY12 Down F	CGATAATTGTTGGGATTCCATTGTTGG
1-28 jm dTY12 Down-2uori R	TGTTCTACAAAATGAAGCACAGATGCTTCGTTCTATCTTCACGGAAAGAATTGCCC
1-29 jm ori-dHO F	TCAGGGGGGCGGAGCCTATGGAAAAACGCCGGTCTTTTGGGGTGTAACGCC
1-30 jm dHO Up-LEU2 R	GTATAGATAGAATTGATTGCTGCTTATGAGG
1-31 jm LEU2-dHO Down F	CTGTCGCCGAAGAAGTTAAGAAAATCCCTTAACTAGAATGCTGGAGTAGAAATACGC
1-32 jm dHO Down- 2uori R	TGTTCTACAAAATGAAGCACAGATGCTTCGTTTTATCTCTAGGTGTTGGTATGCAAGG
1-33 Misa OYE3-TEF1p F	GCGCCAAATCACGGATGTGGAAAACTGATCACGTGCTTCCGCGAATCCTTACATCACACC
1-34 Misa ADH7-TEF1p F	ATGCTGTAGATCAGGGACTATGCGAGCGACAAGTCAGACCGCGAATCCTTACATCACACC
1-35 Misa URA3-TEF1p F	CATCAAAGAAGGTTAATGTGGCTGTGGTTTCAGGGTCCCCGCGAATCCTTACATCACACC
1-36 Misa TY12-TEF1p F	CCAAAATATGGATATCGAGATGTATTTGATGAATAATTCCGCGAATCCTTACATCACACC
1-37 Misa HO-TEF1p F	TCCTCATAAGCAGCAATCAATTCTATCTATACTTTAAACCGCGAATCCTTACATCACACC
1-38 jm dURA3-2uori Trc R	GTTTCGATTGTTTTACGTTTGAGGC
1-39 jm dTY12-2uori Trc R	CTATCTTCACGGAAAGAATTGCCC
1-40 jm ori-dHO Trc F	GTCTTTTGGGGTGTAACGCC
1-41 jm ori-dURA3 Trc F	GAGACTATTTTCATTGACCGAATCAGAG
1-42 jm ori-dTY12 Trc F	ATCTATTAGCTGAACACGGTATCGC
1-43 jm dHO-2uori Trc R	TTATCTCTAGGTGTTGGTATGCAAGG
1-44 jm Ura3 Check 3 F	GCGGATCAGACGGAGTACTTGTC
1-45 jm Ura3 Check 3 R	GGCAAATGTACTCTCGCAGAAGG
1-46 jm Ty12 Check 3 F	ATCCAAGGTATAATAGCGGGTGTTG
1-47 jm Ty12 Check 3 R	GGCACCTTTATTTTTCTGCGAGGG
1-48 jm Ho Check 3 F	CTTGAGGGCACAAAATGTCCAGG
1-49 jm Ho Check 3 R	CCAAAGGTCCAAAAGTTGTTGTCTGAC
1-50 jm 2uori-Leu2 F	CTTCAATGCTATCATTTCCTTTGATATTGGATCGGATTTTCTTAACTTCTTCGGCGACAG
1-51 jm Leu2p-Amp R	AGAAAAATAAACAAATAGGGGTTCCGCGCTAACCATTATTTTTTTCCTCAACATAACGAG
1-52 jm 2uori-Trp F	TCATCCTTCAATGCTATCATTTCCTTTGATATTGGATCCAGGCAAGTGCACAAACAATAC
1-53 jm Trp-Amp R	ATTTAGAAAAATAAACAAATAGGGGTTCCGCGCGCATACATTATACGAAGTTATAACGAC
1-54 Misa ADH7 up 4 F	CGAATTGGGTGTTTACGTCTCCG
1-55 jm Ty12-ADH2p F	ATATGGATATCCGAGATGTATTTGATGAATAATTGCCGCAAAACGTAGGGGCAAACAA
1-56 jm Ho-ADH2 F	ATAAGCAGCAATCAATTCTATCTATACTTTAAAGCCGCAAAACGTAGGGGCAAACAAACG

1-57 jm SPG5t-Ty12 R	CCAACAATGGAATCCCAACAATTATCGAATTAGCTTATTTTCTGCCGAATTTTCATGAAG
1-58 jm SPG5t-Ho R incorrect	AAAAGTTGTATGTAATAAAAGTAAAATTTAATGCTTATTTTCTGCCGAATTTTCATGAAG
1-59 jm SPG5t-Ho' R	GCGTATTTCTACTCCAGCATTCTAGTTAAGGCTTATTTTCTGCCGAATTTTCATGAAG
1-60 jm HO DownHM F	CTTAACTAGAATGCTGGAGTAGAAATACGC
1-61 jm LAMT-6xHis-PRM9t	TTAATGATGATGATGATGATGGCTGCCATTACCCTTCCTCTTCAAGACCAAG
1-62 jm SLS-6xHis-PRM9t	TTAATGATGATGATGATGATGGCTGCCACTTTCCAACTTCTTATAGATGACGTGAGAACC
1-63 jm STR-6xHis-PRM9t	TTAATGATGATGATGATGATGGCTGCCTGAAGAAACGTAGGAGTTACCCTTGTTATCATG
1-64 jm 6xHis-PRM9t	GGCAGCCATCATCATCATCATTAAGACAGAAGACGGGAGACACTAGCAC
1-65 jm ADH2p-PRM9t	ATCAACTATCAACTATTAACTATATCGTAATACCGGACAGAAGACGGGAGACACTAGCAC
1-66 jm ADH2p-LAMT	TATCAACTATTAACTATATCGTAATACCATGGTTGCTACTATCGATTCTATTGAAATGCC
1-67 jm ADH2p-SLS	CTATCAACTATTAACTATATCGTAATACCATGGAAATGGATATGGATACTATCAGAAAGG
1-68 jm ADH2p-STR	ATCAACTATTAACTATATCGTAATACCATGGCTAATTTCTCTGAATCTAAGTCTATGATG
1-69 jm ori-TEF1p F	GATGCTCGTCAGGGGGGCGGAGCCTATGGAAAAACGCCCCGCGAATCCTTACATCACACC
1-70 jm PRM9t.PCK1p F	TTCGGAAAATACGATGTTGAAAATGCCCAATAGGAAAAAACCGAGCTTCCTTTCATCCGG
1-71 jm SPG5t.TDH3p R	GTGGATGCCAGGAATAAACTGTGCTTATTTTCTGCCGAATTTTCATGAAGTTTTTATGCG
1-72 jm CYC1t-OYE3	TTATTCCCTTTGAACAGCGCGCGGGCACGAGAAAGCGCAAATTAAAGCCTTCGAGCGTCC
1-73 jm AmpR-TRP1p	TTAGAAAAATAAACAAATAGGGGTTCCGCGCAATTCGGTCGAAAAAAGAAAAGGAGAGGG
1-74 jm 2uori-URA3	TTCAATGCTATCATTTCCTTTGATATTGGATCGATCCGATGATAAGCTGTCAAACATGAG
1-75 jm URA3-AmpR	TATTTAGAAAAATAAACAAATAGGGGTTCCGCGCATGTCGAAAGCTACATATAAGGAACG
1-76 jm PRM9t-OYE3	CCCTTTGAACAGCGCGCGGGCACGAGAAAGCGGCATTTTCAACATCGTATTTTCCGAAGC
1-77 jm ADH2p-Ca565	ATCAACTATTAACTATATCGTAATACCATGGAGATACAAATGGATGTGCTATACAAGTCC
1-78 jm Ca565-SPG5t	TGGTAATAGCGCGATGAAACAACGTCTTTGCTAGCACTTGATTTTCCTAAAGACGACCTG
1-79 jm ADH2p-Ca610	CAACTATCAACTATTAACTATATCGTAATACCATGAAGATGGAAGTCATGCATATGTCAG
1-80 jm Ca610-SPG5t	AATAGCGCGATGAAACAACGTCTTTGCTAACACTTAAGACGTCTAAAAATTACTTGAGCG
1-81 jm MLS1p-Ca565	GTAAAAGCACATAAAAGAATTAAGAAAATGGAGATACAAATGGATGTGCTATACAAGTCC
2-1 jm MLS1p-Ca610	AAGTAGTAAAAGCACATAAAAGAATTAAGAAAATGAAGATGGAAGTCATGCATATGTCAG
2-2 jm bay_ADH2p F	GATCCAGTTCTCCAGTGACACAGCC
2-3 jm bay_ADH2p R	TTTGTATTGTATTTTGAGGATAGAGTTGACAG

2-4 jm para_ADH2p F	TAGTCTTATCTAAAAATTGCCTTTATAGTCCG
2-5 jm para_ADH2p R	AGTGTATTATAATATAATTGACAGTTGACAG
2-6 jm ADH1t F	GCGAATTTCTTATGATTTATG
2-7 jm ADH1t F 2	CCACACCTCTACCGGCATGC
2-8 jm TDH2t F	ATTTAACTCCTTAAGTTACTTTAATG
2-9 jm TDH2t R	GCGAAAAGCCAATTAGTGTG
2-10 jm CPS1t-bay_ADH2p R	CAGATAAAGGCTGTGTCACTGGAGAACTGGATCATTTGACACTTGATTTGACACTTCTTT
2-11 jm bay_ADH2p-SLS F	TCAACTCTATCCTCAAAATACAATACAAAATGGAAATGGATATGGATACTATCAGAAAGG
2-12 jm SLS-ADH1t R	AAATCATAAATCATAAGAAATTCGCCTAACTTTCCAACTTCTTATAGATGACGTGAGAAC
2-13 jm ADH1t-para_ADH2p R	TGGAGAGACGGACTATAAAGGCAATTTTTAGATAAGACTAGCATGCCGGTAGAGGTGTGG
2-14 jm para_ADH2p-STR F	CAACTGTCAATTATATTATAATACACTATGGCTAATTTCTCTGAATCTAAGTCTATGATG
2-15 jm STR-TDH2t R	TCATTAAAGTAACTTAAGGAGTTAAATTTATGAAGAAACGTAGGAGTTACCCTTGTTATC
2-16 jm TDH2t-2u ori R	TGCATTTTTGTTCTACAAAATGAAGCACAGATGCTTCGTTGCGAAAAGCCAATTAGTGTG
2-17 jm OYE3-PCK1p F	AAATCACGGATGTGGAAAACTGATCACGTGCTTCAATAGGAAAAAACCGAGCTTCCTTTC
2-18 jm OYE3-ICL1p F	ATGTGGAAAACTGATCACGTGCTTATTTATTGAAAAGTAAATATCTCGTAACCCGGATGC
2-19 jm ori-bay_ADH2p F	CTCGTCAGGGGGGCGGAGCCTATGGAAAAACGCCGGATCCAGTTCTCCAGTGACACAGCC
2-20 jm ADH1t-2uori R	TGCATTTTTGTTCTACAAAATGAAGCACAGATGCTTCGTTGCATGCCGGTAGAGGTGTGG
2-21 jm ori-para_ADH2p F	GGGGGGCGGAGCCTATGGAAAAACGCCGTAGTCTTATCTAAAAATTGCCTTTATAGTCCG
2-22 jm Ca565-PRM9t R	AGTTGTGTGCTAGTGTCTCCCGTCTTCTGTCTAGCACTTGATTTTCCTAAAGACGACCTG
2-23 jm Ca610-PRM9t R	GTGTGCTAGTGTCTCCCGTCTTCTGTCTAACACTTAAGACGTCTAAAAATTACTTGAGCG
2-24 jm CPS1t-para_ADH2p F	AAAAGAAGTGTCAAATCAAGTGTCAAATTAGTCTTATCTAAAAATTGCCTTTATAGTCCG
2-25 jm Ca565-CPS1t	AAAAAATCTTTGACTATTCAATCATTGCGCCTAGCACTTGATTTTCCTAAAGACGACCTG
2-26 jm Ca610-CPS1t	AATCTTTGACTATTCAATCATTGCGCCTAACACTTAAGACGTCTAAAAATTACTTGAGCG
2-27 jm MLS1p-GPH1	AAAGTAGTAAAAGCACATAAAAGAATTAAGAAAATGCCGCCAGCTAGTACTAGTACTACC
2-28 jm GPH1-CPS1t	AAAAAAATCTTTGACTATTCAATCATTGCGCCTAAGTCACTGGTTCAACGTTCCAAATGG
2-29 jm MLS1p-UGP1	GTAAAAGCACATAAAAGAATTAAGAAAATGTCCACTAAGAAGCACACCAAAACACATTCC
2-30 jm UGP1-CPS1t	TCTTTGACTATTCAATCATTGCGCTCAATGTTCCAAGATTTGCAAATTACCAGTAACGAC
2-31 jm TY12.PCK1p F	ATATGGATATCGAGATGTATTTGATGAATAATTCAATAGGAAAAAACCGAGCTTCCTTTC
2-32 jm IDP1t.TY12 R	CTTTACCAACAATGGAATCCCAACAATTATCGAATTAGATGGTAATGATCCGAACTTGGG

2-33 jm bay_ADH2-STR F	AACTCTATCCTCAAAATACAATACAAAATGGCTAATTTCTCTGAATCT AAGTCTATGATG
2-34 jm STR-ADH1t R	AAAAATCATAAATCATAAGAAATTCGCTTATGAAGAAACGTAGGAGTT ACCCTTGTTATC
2-35 jm PRM9t-HO R--NOT GOOD	ACATACAACTTTTTAAACTAATATACACATTGGCATTTTCAACATCGTA TTTTCCGAAGC
2-36 jm PGK1p-INO2 F	CATCAAGGAAGTAATTATCTACTTTTTACAACAAATATATGCAACAAG CAACTGGGAACG
2-37 jm INO2-SPG5t R	GGTAATAGCGCGATGAAACAACGTCTTTGCTCAGGAATCATCCAGTA TGTGCTGTAGTGC
2-38 jm PGK1p R	ATATTTGTTGTAAAAAGTAGATAATTACTTCCTTG
2-39 jm PRM9t.HO R	GGCGTATTTCTACTCCAGCATTCTAGTTAAGGGCATTTTCAACATCGT ATTTTCCGAAGC
2-40 jm TY12-PGK1p F	CCAAAATATGGATATCGAGATGTATTTGATGAATAATTAGGCATTTGC AAGAATTACTCG
2-41 jm Ho-ADH2 F	CCTCATAAGCAGCAATCAATTCTATCTATACTTTAAACAAAACGTAGG GGCAAACAAACG
2-42 jm SPG5t R	GCTTATTTTCTGCCGAATTTTCATGAAGTTTTTATGCG
2-43 jm IDP1t-PCK1p R	CGCGCCGGATGAAAGGAAGCTCGGTTTTTTCCTATTGGATGGTAATG ATCCGAACTTGGG
2-44 jm ori-ICL1p F	GGCGGAGCCTATGGAAAAACGCCGATTTATTGAAAAGTAAATATCTC GTAACCCGGATGC
2-45 jm PTR2 4 F	ATGATCGGACAAGTTGTAGCGTTCCGGTTTTAGAGCTAGAAATAGCA AGTTAAAATAAGG
2-46 jm PTR2 2 R	TTTCTAGCTCTAAAACCGGAACGCTACAACTTGTCCGATCATTTATCT TTCACTGCGGAG
2-47 jm ICL1p-7DLH F	AGCATAACATAACAAAAAGTCAACGAAAAATGGAACTGAACTTTAAGT CTATCATCTTTC
2-48 jm PCK1p-SLS F	AACTAATTATTCCATAATAAAATAACAACATGGAAATGGATATGGATA CTATCAGAAAGG
2-49 jm SLS-CPS1t R	ATCTTTGACTATTCAATCATTGCGCCTAACTTTCCAACTTCTTATAGAT GACGTGAGAAC
2-50 jm DAL5 4 F	ATGATCGATAGTACCGTGCTTAGAACGTTTTAGAGCTAGAAATAGCA AGTTAAAATAAGG
2-51 jm DAL5 2 R	TTTCTAGCTCTAAAACGTTCTAAGCACGGTACTATCGATCATTTATCT TTCACTGCGGAG
2-52 jm TY12-ICL1p F	TCGAGATGTATTTGATGAATAATTATTTATTGAAAAGTAAATATCTCGT AACCCGGATGC
2-53 jm ADH1t-TY12 R	AGCCTTTACCAACAATGGAATCCCAACAATTATCGAATTAGCATGCC GGTAGAGGTGTGG
2-54 jm MLS1p-Lj7DLH F	GTAGTAAAAGCACATAAAAGAATTAAGAAAATGATGATGAGCTATAAC TTAATCGGTGGC
2-55 jm Lj7DLH-PRM9t R	GTTGTGTGCTAGTGTCTCCCGTCTTCTGTCTTATATTTTATGTAAAAT AAGATGTGCCCC
2-56 jm MLS1p-Rs7DLH F	TAAACAAAGTAGTAAAAGCACATAAAAGAATTAAGAAAATGGAAGTCT CCTTCAAAAGCG
2-57 jm Rs7DLH-PRM9t R	AAGTTGTGTGCTAGTGTCTCCCGTCTTCTGTCCTACAGCTTATGTAA AATCAGGTGAGCC
2-58 jm MLS1p-Ti17_7DLH F	CAAAGTAGTAAAAGCACATAAAAGAATTAAGAAAATGGAGGCAAACT TCAAACTAGTCGC
2-59 jm Ti17_7DLH-PRM9t R	AAGTTGTGTGCTAGTGTCTCCCGTCTTCTGTCCTAAAGCTTGTGTAA GATTAGATGAGCC

2-60 jm MLS1p-Ti18_7DLH F	CAAAGTAGTAAAAGCACATAAAAGAATTAAGAAAATGGAAGCCAACTTTAAATTGGTGGC
2-61 jm Ti18_7DLH-PRM9t R	AAGTTGTGTGCTAGTGTCTCCCGTCTTCTGTCCTACAGTTTCCTAAGGATAAGGTGTGCC
2-62 jm MLS1p-Ug7DLH F	TAAACAAAGTAGTAAAAGCACATAAAAGAATTAAGAAAATGGGCGTCAACTTTAGTAGCG
2-63 jm Ug7DLH-PRM9t R	GTGTGCTAGTGTCTCCCGTCTTCTGTCCTAAAGCAACATTTTCTGATTTTCTAACTTGTG
2-64 jm MLS1p-Lj7DLH F	AAAGTAGTAAAAGCACATAAAAGAATTAAGAAAATGATGATGAGCTATAACTTAATCGGT
2-65 jm Lj 7DLH R	TTATATTTTATGTAAAATAAGATGTGCC
2-66 jm Lj 7DLH-PRM9t F	AATTTGGGGCACATCTTATTTTACATAAAATATAAGACAGAAGACGGGAGACACTAGCAC
2-67 jm MLS1p-Rs7DLH F	AAACAAAGTAGTAAAAGCACATAAAAGAATTAAGAAAATGGAAGTCTCCTTCAAAAGCGT
2-68 jm MLS1p-Ti18_7DLH F	AACAAAGTAGTAAAAGCACATAAAAGAATTAAGAAAATGGAAGCCAACTTTAAATTGGTG
2-69 jm Ti18_7DLH R	CTACAGTTTCCTAAGGATAAGGTGTG
2-70 jm Ti18_7DLH-PRM9t F	AATACGGGGCACACCTTATCCTTAGGAAACTGTAGGACAGAAGACGGGAGACACTAGCAC
2-71 jm IDP1t-HO R	GGCGTATTTCTACTCCAGCATTCTAGTTAAGATGGTAATGATCCGAACTTGGG
2-72 jm CPS1t-HO R	ATGGCGTATTTCTACTCCAGCATTCTAGTTAAGATTTGACACTTGATTTGACACTTCTTT
2-73 jm ADH2p-CrSGD F	ACAATCAACTATCAACTATTAACTATATCGTAATACCATGGGGAGCAAAGACGACCAATC
2-74 jm CrSGD-SPG5t R	GGTAATAGCGCGATGAAACAACGTCTTTGCCTAATATTTCTGTTTTTTCACCAGTTCCAC
2-75 jm ICL1p-CrGS F	AAAACTCTTAGCATAACATAACAAAAAGTCAACGAAAAATGGCAGGCGAGACGACAAAGC
2-76 jm CrGS-PRM9t R	AAAGTTGTGTGCTAGTGTCTCCCGTCTTCTGTCTCACTCCTCGAACTTCAGAGTATTTCC
2-77 jm PCK1p-CrGO F	CACGCAACTAATTATTCCATAATAAAATAACAACATGGAGTTCTCTTTCTCCTCACCTGC
2-78 jm CrGO-CPS1t R	AAAAAAAATCTTTGACTATTCAATCATTGCGCCTAATCGTTTACTAAGTGGGGTACTAAC
2-79 jm ProGly6x-CNE1 F	GACCCGGTCCAGGGCCCGGACCAGGCCCTGGTATATTAGAGCAACCTCTGAAATTTGTGC
2-80 jm 7-DLGT-ProGly6x	CTGGTCCGGGCCCTGGACCGGGTCCTGGGATAATCAAGGACTTAATGTAATCAACCAGGC
2-81 jm CNE1 F	ATGAAATTTTCTGCGTATTTATGGTGGC
3-1 jm CNE1-IDP1t R	AAGTGGTAGATTGGGCTACGTAAATTCGACTATGTAAATACTACACAACAAAGAACCGAC
3-2 jm dTY12 Up-Down R	AACAATTATCGAATTAcctaggAATTATTCATCAAATACATCTCGATATCCATATTTTGG
3-3 jm dTY12 Up-Down F	GAGATGTATTTGATGAATAATTcctaggTAATTCGATAATTGTTGGGATTCCATTGTTGG
3-4 jm dHO Up-Down R	CCAGCATTCTAGTTAAGcctaggTTTAAAGTATAGATAGAATTGATTGCTGCTTATGAGG
3-5 jm dHO Up-Down F	AATCAATTCTATCTATACTTTAAAcctaggCTTAACTAGAATGCTGGAGTAGAAATACGC
3-6 jm ori-d514c Up	GGGCGGAGCCTATGGAAAAACGCCGGTTTGTTTCTTCTTATCTTCAGCTGCTGAG

3-7 jm d514c Up-Down R	CTTCTAGCTAGATcctaggACAATAGCTTATAATCTGTGTAGTCAAACTATATACTAGGC
3-8 jm d514c Up-Down F	TAAGCTATTGTcctaggATCTAGCTAGAAGTTTTGTAGGTATATGTGATTTAAGATATAG
3-9 jm d514c Down-2u ori R	CTACAAAATGAAGCACAGATGCTTCGTTCATTATCACGTTGTTTGCCACAAGAATTATTG
3-10 jm YDR514C-ADH2p	TATATAGTTTGACTACACAGATTATAAGCTATTGTCGCCGCAAAACGTAGGGGCAAACAA
3-11 jm SPG5t-YDR514C R	CTACAAAACTTCTAGCTAGATCGCTTATTTTCTGCCGAATTTTCATGAAGTTTTTATGCG
3-12 jm Cas9 F	GCAGTGAAAGATAAATGATCCTCGAGGTTTTAGAGCTAGAAATAGCAAGTTAAAATAAGG
3-13 jm Cas9 R	ATTTTAACTTGCTATTTCTAGCTCTAAAACCTCGAGGATCATTTATCTTTCACTGCGGAG
3-14 jm TY12 sgRNA F	CAGTGAAAGATAAATGATCCGAATGTGACTGAGCAGTTTGGGTTTTAGAGCTAGAAATAG
3-15 jm TY12 sgRNA R	CTATTTCTAGCTCTAAAACCCAAACTGCTCAGTCACATTCGGATCATTTATCTTTCACTG
3-16 jm HO sgRNA F	GCAGTGAAAGATAAATGATCGTAAGGCTTCATTATGGAGAGGTTTTAGAGCTAGAAATAG
3-17 jm HO sgRNA R	CTATTTCTAGCTCTAAAACCTCTCCATAATGAAGCCTTACGATCATTTATCTTTCACTGC
3-18 jm YDR514C sgRNA F	CAGTGAAAGATAAATGATCCGTGATGAATTGTGAACCTGGGGTTTTAGAGCTAGAAATAG
3-19 jm YDR514C sgRNA R	CTATTTCTAGCTCTAAAACCCCAGGTTCACAATTCATCACGGATCATTTATCTTTCACTG
3-20 jm CPS1t-YDR514C R	ATCACATATACCTACAAAACTTCTAGCTAGATCATTTGACACTTGATTTGACACTTCTTT
3-21 jm EGH1 sgRNA F	CAGTGAAAGATAAATGATCCGCACAGTATAAGGTCCATAGGGTTTTAGAGCTAGAAATAG
3-22 jm EGH1 sgRNA R	CTATTTCTAGCTCTAAAACCCTATGGACCTTATACTGTGCGGATCATTTATCTTTCACTG
3-23 jm PDR1 sgRNA F	CAGTGAAAGATAAATGATCCGTTGGTTGTTCCACTGCGCAGGTTTTAGAGCTAGAAATAG
3-24 jm PDR1 sgRNA R	CTATTTCTAGCTCTAAAACCTGCGCAGTGGAACAACCAACGGATCATTTATCTTTCACTG
3-25 jm PDR5 sgRNA F	CAGTGAAAGATAAATGATCCGTACTTCTTGGCCTTATCGGGGTTTTAGAGCTAGAAATAG
3-26 jm PDR5 sgRNA R	CTATTTCTAGCTCTAAAACCCCGATAAGGCCAAGAAGTACGGATCATTTATCTTTCACTG
3-27 jm Ty12-ADH2p F	AAATATGGATATCGAGATGTATTTGATGAATAATTCGCCGCAAAACGTAGGGGCAAACAA
3-28 jm SPG5t-Ty12 R	CAACAATGGAATCCCAACAATTATCGAATTACGCTTATTTTCTGCCGAATTTTCATGAAG
3-29 jm YDR514C Up R	ACAATAGCTTATAATCTGTGTAGTCAAACTATATACTAGGC
3-30 jm YDR514C Down F	ATCTAGCTAGAAGTTTTGTAGGTATATGTGATTTAAGATATAG
3-31 jm CEN-HIS3 F	TAAATTATAATTATTTTTATAGCACGTGATTCGAGTTCAAGAGAAAAAAAAAGAAAAAGC
3-32 jm CEN/ARS ori R	ATCACGTGCTATAAAAATAATTATAATTTA
3-33 jm CPS1t-CEN F	ATAAAAAAAAAAAGAAGTGTCAAATCAAGTGTCAAATGTAACTTACACGCGCCTCGTATC
3-34 jm YDR514C Up F	GTTTGTTTCTTCTTATCTTCAGCTGCTGAG

3-35 jm YDR514C Down R	CATTATCACGTTGTTTGCCACAAGAATTATTG
3-36 jm EGH1 seq F	CTGCAAAGCCATTCCTATCCACC
3-37 jm EGH1 seq R	GATTGATTGTGCACGGTTTTCCC
3-38 jm PDR1 seq F	GAACGGTGTACATATTGAGACGGG
3-39 jm PDR1 seq R	GATGATATCGAAGATGGGGTTGAAGG
3-40 jm PDR5 seq F	AATTTCTTACAGCGGCTACTCAGG
3-41 jm PDR5 seq R	AAACCCGATATTATTTCGCAGTCTCC
3-42 jm PAH1 sgRNA F	CAGTGAAAGATAAATGATCCGAACGATGGCTGCGTTAAGGGGTTTTAGAGCTAGAAATAG
3-43 jm PAH1 sgRNA R	CTATTTCTAGCTCTAAAACCCCTTAACGCAGCCATCGTTCGGATCATTTATCTTTCACTG
3-44 jm iCas9 Seq 1	AAGACAAGAAGCATGAACGTCATCC
3-45 jm iCas9 Seq 2	ACCCATCAAATTCACTTGGGTGAGC
3-46 jm iCas9 Seq 3	CACAAGTGTCTGGACAAGGCG
3-47 jm iCas9 Seq 4	CCAAAACTTGAATCGGAGTTTGTCTATGG
3-48 jm Leu2 sgRNA F	CAGTGAAAGATAAATGATCCGTGCTGTGGGTGGTCCTAAAGGTTTTAGAGCTAGAAATAG
3-49 jm Leu2 sgRNA R	CTATTTCTAGCTCTAAAACCTTTAGGACCACCCACAGCACGGATCATTTATCTTTCACTG
3-50 jm TEF1p.IO F	GCATAGCAATCTAATCTAAGTTTTAATTACAAAATGGCTACCATTACGTTTGATTCCTTG
3-51 jm IO.PRM9t R	GTAAAGTTGTGTGCTAGTGTCTCCCGTCTTCTGTCTCAGATGTGGACCCTCTTCTTTGGG
3-52 jm PGK1 R	ATATTTGTTGTAAAAAGTAGATAATTACTTCCTTG
3-53 jm PGK1p.7-DLH F	AGTAATTATCTACTTTTTACAACAAATATATGGAACTGAACTTTAAGTCTATCATCTTTC
3-54 jm 7-DLH.SPG5t	GGTAATAGCGCGATGAAACAACGTCTTTGCTCACAACTTATGCAAAATTAAATGAGCACC
3-55 jm TDH3p R	TTTGTTTGTTTATGTGTGTTTATTCGAAAC
3-56 jm TDH3p.SLS F	TTTCGAATAAACACACATAAACAAACAAAATGGAAATGGATATGGATACTATCAGAAAGG
3-57 jm SLS.CYC1t R	AAGCGTGACATAACTAATTACATGACTAACTTTCCAACTTCTTATAGATGACGTGAGAAC
3-58 jm CYC1t.CEN R	tccatcattaaaaGATACGAGGCGCGTGTAAGTTACGCAAATTAAAGCCTTCGAGCGTCC
3-59 jm CYC1t.CEN F	TGAGAAGGTTTTGGGACGCTCGAAGGCTTTAATTTGCGTAACTTACACGCGCCTCGTATC
3-60 jm ori-TRP1 Up F	GGGCGGAGCCTATGGAAAAACGCCGAGTTAGAGGCGGTGGAGATATTCC
3-61 jm TRP1 Up-Down R	AATACTTAAATAAATACTACTCAGTcctaggTTCACCAATGGACCAGAACTACCT
3-62 jm TRP1 Up-Down F	AGGTAGTTCTGGTCCATTGGTGAAcctaggACTGAGTAGTATTTATTTAAGTATT
3-63 jm TRP1 Down-2u R	TGCATTTTTGTTCTACAAAATGAAGCACAGATGCTTCGTTCTGATGGTGTTTATGCAAAG
3-64 jm TRP1-ADH2p	ATTAATTTCACAGGTAGTTCTGGTCCATTGGTGAACGCCGCAAAACGTAGGGGCAAACAA
3-65 jm CPS1t-TRP1 R	GCACAAACAATACTTAAATAAATACTACTCAGTATTTGACACTTGATTTGACACTTCTTT

3-66 jm ori-URA3 Up F	GGGCGGAGCCTATGGAAAAACGCCGGGACTATTAGATGAAATTTCA TCAATGGGTGC
3-67 jm URA3 Up-Down R	GGATAGTTCCTTTTTATAAAGGCCATGAAGCcctaggCTGAAACCACAG CCACATTAACC
3-68 jm URA3 Up-Down F	GGTTAATGTGGCTGTGGTTTCAGcctaggGCTTCATGGCCTTTATAAAA AGGAACTATCC
3-69 jm URA3 Down-2u R	TGCATTTTTGTTCTACAAAATGAAGCACAGATGCTTCGTTGTCGGACT CCGATGGGAACG
3-70jm URA3-ADH2p F	ACCATCAAAGAAGGTTAATGTGGCTGTGGTTTCAGCGCCGCAAAAC GTAGGGGCAAACAA
3-71 jm CPS1t-URA3 R	TGGATAGTTCCTTTTTATAAAGGCCATGAAGCCATTTGACACTTGATT TGACACTTCTTT
3-72jm ori-HIS3 Up F	GGGCGGAGCCTATGGAAAAACGCCGCTTGATCTCCTTTAGCTTCTC GACGTG
3-73jm HIS3 Up-Down R	ATACCACTTGCCACCTATCACCACcctaggCTTTGCCTTCGTTTATCTT GCCTGC
3-74jm HIS3 Up-Down F	TGAGCAGGCAAGATAAACGAAGGCAAAGcctaggGTGGTGATAGGTG GCAAGTGG
3-75jm HIS3 Down-2u R	TTTTTGTTCTACAAAATGAAGCACAGATGCTTCGTTATAACAATCCGT CCAATGGAGGTG
3-76jm HIS3-ADH2p F	AAAAAATGAGCAGGCAAGATAAACGAAGGCAAAGCCGCCGCAAAAC GTAGGGGCAAACAA
3-77 jm CPS1t-HIS3 R	CTTACGGAATACCACTTGCCACCTATCACCACCATTTGACACTTGATT TGACACTTCTTT
3-78 jm TRP1-Leu2 F	TTCACAGGTAGTTCTGGTCCATTGGTGAATAACCATTATTTTTTTCCT CAACATAACGAG
3-79 jm TRP1-Leu2 R	ATACTTAAATAAATACTACTCAGTCTTAAGCAAGGATTTTCTTAACTTC TTCGGCGACAG
3-80 jm URA3-Leu2 F	AAAGAAGGTTAATGTGGCTGTGGTTTCAGTAACCATTATTTTTTTCCT CAACATAACGAG
3-81 jm URA3-Leu2 R	TCCTTTTTATAAAGGCCATGAAGCCTTAAGCAAGGATTTTCTTAACTT CTTCGGCGACAG
4-1 jm HIS3-Leu2 F	ATGAGCAGGCAAGATAAACGAAGGCAAAGTAACCATTATTTTTTTCC TCAACATAACGAG
4-2 jm HIS3-Leu2 R	ATACCACTTGCCACCTATCACCACCTTAAGCAAGGATTTTCTTAACTT CTTCGGCGACAG
4-3 jm 2u ori-Leu2 F	tatcatttcctttgatattggatcCTTAAGCAAGGATTTTCTTAACTTCTTCGGCGA CAG
4-4 jm ADH2p-Redox 1 F	ataCAATCAACTATCAACTATTAACTATATCGTAATACCATGGCGGACC GTGTCAAGACG
4-5 jm Redox 1-SPG5t R	CTTCTTGGTAATAGCGCGATGAAACAACGTCTTTGCTCACACGGCTA CCGTTGCATTGCC
4-6 jm ICL1p-Redox 2 F	CTTAGCATAACATAACAAAAAGTCAACGAAAAATGGAGAAACAAGTA GAAATCCCCGAGG
4-7 jm Redox 2-PRM9t R	CCTGGTAAAGTTGTGTGCTAGTGTCTCCCGTCTTCTGTCTCACAGGT CACCGTCCCACAG
4-8 jm PCK1p-SAT F	ACGCAACTAATTATTCCATAATAAAATAACAACATGGCACCTCAGATG CAAATACTTTCC
4-9 jm SAT-CPS1t R	ATCTTTGACTATTCAATCATTGCGCCTCAGTTACTAAAATCCGTATCT AACAAACTCAGG
4-10 jm SPG5t-TRP1 R	CACAAACAATACTTAAATAAATACTACTCAGTGCTTATTTTCTGCCGA ATTTTCATGAAG
4-11 jm PRM9t-TRP1 R	ACAAACAATACTTAAATAAATACTACTCAGTGGCATTTTCAACATCGT ATTTTCCGAAGC

4-12 jm SPG5t-URA3 R	TGGATAGTTCCTTTTTATAAAGGCCATGAAGCGCTTATTTTCTGCCGAATTTTCATGAAG
4-13 jm PRM9t-URA3 R	GGATAGTTCCTTTTTATAAAGGCCATGAAGCGGCATTTTCAACATCGTATTTTCCGAAGC
4-14 jm SPG5t-HIS3 R	CTTACGGAATACCACTTGCCACCTATCACCACGCTTATTTTCTGCCGAATTTTCATGAAG
4-15 jm PRM9t-HIS3 R	TTACGGAATACCACTTGCCACCTATCACCACGGCATTTTCAACATCGTATTTTCCGAAGC
4-16 jm SPG5t-CEN F	GCATAAAAACTTCATGAAAATTCGGCAGAAAATAAGCGTAACTTACACGCGCCTCGTATC
4-17 jm SPG5t-CEN R	TCATTAAAAGATACGAGGCGCGTGTAAGTTACGCTTATTTTCTGCCGAATTTTCATGAAG
4-18 jm ori-ICL1p F	GGCGGAGCCTATGGAAAAACGCCGATTTATTGAAAAGTAAATATCTCGTAACCCGGATGC
4-19 jm ori-ICL1p R	AAGCATCCGGGTTACGAGATATTTACTTTTCAATAAATCGGCGTTTTTCCATAGGCTCCG
4-20 jm PRM9t-CEN F	TATGCAACGCTTCGGAAAATACGATGTTGAAAATGCCGTAACTTACACGCGCCTCGTATC
4-21 jm PRM9t-CEN R	CATTAAAAGATACGAGGCGCGTGTAAGTTACGGCATTTTCAACATCGTATTTTCCGAAGC
4-22 jm ori-PCK1p F	CGTCAGGGGGGCGGAGCCTATGGAAAAACGCCGCAATAGGAAAAAACCGAGCTTCCTTTC
4-23 jm ori-PCK1p R	CCGCGCCGGATGAAAGGAAGCTCGGTTTTTTCCTATTGCGGCGTTTTTCCATAGGCTCCG
4-24 jm ADH2p-Redox1 R	GACCGTCTTGACACGGTCCGCCATGGTATTACGATATAGTTAATAGTTGATAGTTGATTG
4-25 jm Redox1-SPG5t F	TTCGTGCTTGACATCGGCAATGCAACGGTAGCCGTGTGAGCAAAGACGTTGTTTCATCGC
4-26 jm ICL1p-Redox2 R	TCGGGGATTTCTACTTGTTTCTCCATTTTTCGTTGACTTTTTGTTATGTTATGCTAAGAG
4-27 jm Redox2-PRM9t F	AAAGCCCGGAGGAACTGTGGGACGGTGACCTGTGAGACAGAAGACGGGAGACACTAGCAC
4-28 jm PCK1p-SAT R	GAAAGTATTTGCATCTGAGGTGCCATGTTGTTATTTTATTATGGAATAATTAGTTGCGTG
4-29 jm SAT-CPS1t F	CTGAGTTTGTTAGATACGGATTTTAGTAACTGAGGCGCAATGATTGAATAGTCAAAGATT
4-30 jm SPG5T-PCK1p F	AAAAACTTCATGAAAATTCGGCAGAAAATAAGCCAATAGGAAAAAACCGAGCTTCCTTTC
4-31 jm SPG5t-PCK1p R	CGGATGAAAGGAAGCTCGGTTTTTTCCTATTGGCTTATTTTCTGCCGAATTTTCATGAAG
4-32 jm Leu2 sgRNA F n	GCAGTGAAAGATAAATGATCGTGCTGTGGGTGGTCCTAAAGTTTTAGAGCTAGAAATAGC
4-33 jm Leu2 sgRNA R n	GCTATTTCTAGCTCTAAAACTTTAGGACCACCCACAGCACGATCATTTATCTTTCACTGC
4-34 jm Cas9 F n	TCGAAACTTCTCCGCAGTGAAAGATAAATGATCTCGAGTTTTAGAGCTAGAAATAGCAAG
4-35 jm Cas9 R n	TTATTTTAACTTGCTATTTCTAGCTCTAAAACTCGAGATCATTTATCTTTCACTGCGGAG
4-36 jm NcoI-CrSGD F	GAAATAATTTTGTTTAACTTTAAGAAGGAGATATACCATGGGGAGCAAAGACGACCAATC
4-37 jm CrSGD-XhoI R	TTAGCAGCCGGATCTCAGTGGTGGTGGTGGTGGTGCTCGAGATATTTCTGTTTTTTCACC
4-38 jm NcoI-CrGS F	AGAAATAATTTTGTTTAACTTTAAGAAGGAGATATACCATGGCAGGCGAGACGACAAAGC

4-39 jm CrGS-XhoI R	AGCCGGATCTCAGTGGTGGTGGTGGTGGTGCTCGAGCTCCTCGAACTTCAGAGTATTTCC
4-40 jm trSGD-SPG5t R	CCTTCTTGGTAATAGCGCGATGAAACAACGTCTTTGCCTACGCCGTGTTTGTTACGAAGC
4-41 jm TRP1-Leu2 2 F	ACACAAAGGCAGCTTGGAGTATGTCTGTTATTAATTTCACAGGTAGTTCTGGTCCATTGG
4-42 jm Leu2-TRP1 2 R	CAAAAGGCCTGCAGGCAAGTGCACAAACAATACTTAAATAAATACTACTCAGTCTTAAGC
4-43 jm URA3-Leu2 2 F	GAGGCATATTTATGGTGAAGGATAAGTTTTGACCATCAAAGAAGGTTAATGTGGCTGTGG
4-44 jm Leu2-URA3 2 R	AATACTGTTACTTGGTTCTGGCGAGGTATTGGATAGTTCCTTTTTATAAAGGCCATGAAG
4-45 jm HIS3-Leu2 2 F	ATATAAAGTAATGTGATTTCTTCGAAGAATATACTAAAAAATGAGCAGGCAAGATAAACG
4-46 jm Leu2-HIS3 2 R	AGCCATAATATGAAATGCTTTTCTTGTTGTTCTTACGGAATACCACTTGCCACCTATCAC
4-47 jm TRP1-hygR F	TGTTATTAATTTCACAGGTAGTTCTGGTCCATTGGTGAAattaccctgttatccctagac
4-48 jm hygR-TRP1 R	AGTGCACAAACAATACTTAAATAAATACTACTCAGTCCACATTAACCTTCTTTGATGGTc
4-49 jm NdeI-trSGD F	TCATCACAGCAGCGGCCTGGTGCCGCGCGGCAGCCATATGGGGAGCAAAGACGACCAATC
4-50 jm trSGD-XhoI R	TCATCACAGCAGCGGCCTGGTGCCGCGCGGCAGCCATATGGGGAGCAAAGACGACCAATC
4-51 jm NdeI-GS F	ATCATCACAGCAGCGGCCTGGTGCCGCGCGGCAGCCATATGGCAGGCGAGACGACAAAGC
4-52 jm GS-XhoI R	CGGATCTCAGTGGTGGTGGTGGTGGTGCTCGAGTCACTCCTCGAACTTCAGAGTATTTCC
4-53 jm NdeI-trSGD 2 F	AGCGGCCTGGTGCCGCGCGGCAGCCATATGGGGAGCAAAGACGACCAATC
4-54 jm trSGD-XhoI 2 R	TCAGTGGTGGTGGTGGTGGTGCTCGAGCTACGCCGTGTTTGTTACGAAGC
4-55 jm TRP1 Homology 2 R	ACAGATTTTATGTTTAGATCTTTTATGCTTGCTTTTCAAAAGGCCTGCAGGCAAGTGCAC
4-56 jm TRP1 Up F	GAGTTAGAGGCGGTGGAGATATTCC
4-57 jm TRP1 Up R	TTCACCAATGGACCAGAACTACCTGTG
4-58 jm TRP1 Down F	ACTGAGTAGTATTTATTTAAGTATTGTTTGTGCAC
4-59 jm TRP1 Down R	CTGATGGTGTTTATGCAAAGAAACCACTG
4-60 jm URA3 Up F	GGACTATTAGATGAAATTTCATCAATGGGTGC
4-61 jm URA3 Up R	CTGAAACCACAGCCACATTAACCTTC
4-62 jm URA3 Down F	GCTTCATGGCCTTTATAAAAAGGAACTATCC
4-63 jm URA3 Down R	GTCGGACTCCGATGGGAACG
4-64 jm HIS3 Up F	CTTGATCTCCTTTAGCTTCTCGACGTG
4-65 jm HIS3 Up R	CTTTGCCTTCGTTTATCTTGCCTGC
4-66 jm HIS3 Down F	GTGGTGATAGGTGGCAAGTGG
4-67 jm HIS3 Down R	ATAACAATCCGTCCAATGGAGGTG
4-68 jm ADH2p-CaSGD R	TAAGGGTATGGATTGTGCTTCCATGGTATTACGATATAGTTAATAGTTGATAGTTGATTG
4-69 jm CaSGD-SPG5t F	GATCACAAAGAACTTGATAACATCCCGCAAAAGAAGTAGGCAAAGACGTTGTTTCATCGC

4-70 jm ADH2-GsSGD R	TATTGTGCTTGACGGGGTGGCCATGGTATTACGATATAGTTAATAGT TGATAGTTGATTG
4-71 jm GsSGD-SPG5t F	AACCAAGAGACGGACTCTCGTAAGCGTAGTCGTAAGTAGGCAAAGA CGTTGTTTCATCGC
4-72 jm ADH2P-RsSGD R	TGGCTCCGCCTGCGTGTTGTCCATGGTATTACGATATAGTTAATAGT TGATAGTTGATTG
4-73 jm RsSGD-SPG5t F	GAGGCACAGGTGGAACTAGTTAAGAGGCAGAAGACTTAGGCAAAGA CGTTGTTTCATCGC
4-74 jm ADH2p-MsSGD	GGCTGTAGACCTTTTAGCTTCCATGGTATTACGATATAGTTAATAGTT GATAGTTGATTG
4-75 jm MsSGD-SPG5t F	CATGAAGATTTTGTTTCTAAAAAACGTCTTCGTCAGTAGGCAAAGAC GTTGTTTCATCGC
4-76 jm G8H-SPG5t R	GGTAATAGCGCGATGAAACAACGTCTTTGCTTACAAAGTAGATGGAA CAGCTCTCAATGG
4-77 jm ADH2p-HIS3 R	CTTTTTGCTTTTTTCTTTTTTTTTTCTCTTGAACTCGAGCAAAACGTAGG GGCAAACAAACG
4-78 jm ADH2P-HIS3 F	TTTTTCCGTTTGTTTGCCCCTACGTTTTGCTCGAGTTCAAGAGAAAAA AAAAGAAAAAGC
4-79 jm kanMX seq 1	ggcgcgaagcaaaaattacgg
4-80 jm kanMX seq 2	gacgcatgatattactttctgcgc
4-81 jm PCK1p-CrGS F	AACTCACGCAACTAATTATTCCATAATAAAATAACAACATGGCAGGC GAGACGACAAAGC
5-1 jm CipA-CYC1t F	ATAACCGTAACTGGTTCTGCACCCTAGTCATGTAATTAGTTATGTCAC GCTTACATTCAC
5-2 jm TEF1p-Oleosin R	AATACCAGACCTATCTCTATCAGCCATTTTGTAATTAAAACTTAGATT AGATTGCTATGC
5-3 jm CipA 2 F	GACAAAGATATAAAAGATGCAGCATCTAACGGCAAAATAACCGTAAC TGGTTCTGCACCC
5-4 jm Oleosin R 2	TGTTGTTGACCATAAGTCGCGTGAGCACCCCCATAAATACCAGACCT ATCTCTATCAGCC
5-5 jm STR-DockA R	AGAGCCCCCTCCGCCACTCCCGCCCCCACCTGAAGAAACGTAGGA GTTACCCTTGTTATC
5-6 jm DockA-PRM9t F	TTACTATTATCCAGATACCTTTTGAGAGTGATTTAAACAGAAGACGGG AGACACTAGCAC
5-7 jm trSGD-DockB R	TGAAATCTCCAGATCCTCCTCCACCACTCCCTCCCCCTCCCGCCGT GTTTGTTACGAAGC
5-8 jm DockB-SPG5t F	ATTTCATTCATAAACCACCGTATTTTAAATTTAGAATAGGCAAAGACG TTGTTTCATCGC
5-9 jm CrGS-DockC R	GGTGTTAGAACCGCCTCCCCCACTCCCTCCTCCACCCTCCTCGAAC TTCAGAGTATTTCC
5-10 jm DockC-CPS1t F	GATGGAGCTAATAAAAAAGGTATCCAATAACTGAGCGCAATGATTGA ATAGTCAAAGATT
5-11 jm SPG5t-CEN F	GCATAAAAACTTCATGAAAATTCGGCAGAAAATAAGCGTAACTTACA CGCGCCTCGTATC
5-12 jm CEN-IDP1t R	AAGGTTCCCCAAGTTCGGATCATTACCATCATCACGTGCTATAAAAA TAATTATAATTTA
5-13 jm SPG5t-CEN R	TCATTAAAAGATACGAGGCGCGTGTAAGTTACGCTTATTTTCTGCCG AATTTTCATGAAG
5-14 jm CEN-IDP1t F	ATTTAAATTATAATTATTTTTATAGCACGTGATGATGGTAATGATCCGA ACTTGGGGAAC
5-15 jm TRP1 Up-Leu2 R	TTCTCGTTATGTTGAGGAAAAAAATAATGGTTATTCACCAATGGACCA GAACTACCTGTG

5-16 jm Leu2-TRP1 Down F	GAAGTTAAGAAAATCCTTGCTTAAGACTGAGTAGTATTTATTTAAGTATTGTTTGTGCAC
5-17 jm DockB-SPG5t R	TTCTTGGTAATAGCGCGATGAAACAACGTCTTTGCCTATTCTAAATTTAAAATACGGTGG
5-18 jm 514C Up--Leu2 F	GTTTGACTACACAGATTATAAGCTATTGTTAACCATTATTTTTTTCCTCAACATAACGAG
5-19 jm Leu2-514C Down R	AATCACATATACCTACAAAACTTCTAGCTAGATGGATTTTCTTAACTTCTTCGGCGACAG
5-20 jm ADH2p-G8H F	TCAACTATCAACTATTAACTATATCGTAATACCATGGACTACCTGACCATTATTTTGACC
5-21 jm G8H-SPG5t R	TTCTTGGTAATAGCGCGATGAAACAACGTCTTTGCTTACAAAGTAGATGGAACAGCTCTC
5-22 jm ori-IAI11 Up F	GGGCGGAGCCTATGGAAAAACGCCGCATTTATCGAGTGCATTGATGAAGTCC
5-23 jm IAI11 Up-Leu2 R	TATGTTGAGGAAAAAAATAATGGTTAATTTTCTTCATGGCAATTCTACATGTTATAAGTG
5-24 jm IAI11-Leu2 F	ATAACATGTAGAATTGCCATGAAGAAAATTAACCATTATTTTTTTCCTCAACATAACGAG
5-25 jm Leu2-IAI11 R	TATTGCCTATGCATACCTTTTGCAGTTATCCTTAAGCAAGGATTTTCTTAACTTCTTCGG
5-26 jm Leu2-IAI11 Down R	GTCGCCGAAGAAGTTAAGAAAATCCTTGCTTAAGGATAACTGCAAAAGGTATGCATAGGC
5-27 jm IAI11 Down-2u R	TTTTTGTTCTACAAAATGAAGCACAGATGCTTCGTTGGTTGGTTCAGGAGAGGTAGAACC
5-28 jm IAI11 Up-ADH2p F	TACACTTATAACATGTAGAATTGCCATGAAGAAAATGCCGCAAAACGTAGGGGCAAACAA
5-29 jm SPG5t-IAI11 Down R	GTTATTGCCTATGCATACCTTTTGCAGTTATCGCTTATTTTCTGCCGAATTTTCATGAAG
5-30 jm IAI11 Up R	ATTTTCTTCATGGCAATTCTACATGTTATAAGTG
5-31 jm IAI11 Down F	GATAACTGCAAAAGGTATGCATAGGC
5-32 jm MATa F	AGTCACATCAAGATCGTTTATGG
5-33 jm MATalpha R	GCACGGAATATGGGACTACTTCG
5-34 jm MATa R	ACTCCACTTCAAGTAAGAGTTTG
5-35 jm IAI11 UP F	CATTTATCGAGTGCATTGATGAAGTCC
5-36 jm IAI11 Down R	GGTTGGTTCAGGAGAGGTAGAACC
5-37 jm IAI11 Up F 2	GATAGTCTTTAATATAGCGTCCTCGCC
5-38 jm IAI11 Down R 2	CTTCATCGTCATCATCAGCTTGACC
5-39 jm YDR514C Up F 2	TCTACAGGAATTACTGTATTGCTATCTGGC
5-40 jm YDR514C Down R 2	TTTTCATATAAAAAGTCCCAGGACGCC
5-41 jm SGD seq 1	GATTGCCGACGGAAGTAATGG
5-42 jm SGD seq 2	GTATGAGGGCGCTAGTCGGG
5-43 jm CPS1t-IAI11 Down R	AGTTATTGCCTATGCATACCTTTTGCAGTTATCATTTGACACTTGATTTGACACTTCTTT
5-44 jm bay_ADH2-trSTR	AACTGTCAACTCTATCCTCAAAATACAATACAAAATGAAGAAAATCTTCATTGAGTCTCC
5-45 jm CrSGD-G4S-STR R	TTCAGAGAAATTAGCCATAGATCCTCCTCCACCATATTTCTGTTTTTTCACCAGTTCCAC
5-46 jm CrSGD-G4S-STR F	AAACAGAAATATGGTGGAGGAGGATCTATGGCTAATTTCTCTGAATCTAAGTCTATGATG

5-47 jm bay_ADH2-STR R	ATGGAGACTCAATGAAGATTTTCTTCATTTTGTATTGTATTTTGAGGATAGAGTTGACAG
5-48 jm TEF1p-CoCthem R	AATTTCCACAACCACCCCATCAGAGGCTTTGTAATTAAAACTTAGATTAGATTGCTATGC
5-49 jm TEF1p-CoCthem F	AGAAAGCATAGCAATCTAATCTAAGTTTTAATTACAAAGCCTCTGATGGGGTGGTTGTGG
5-50 ry PAH1 Up F	TAGAGTCCAAACTCAACAGCCGC
5-51 ry PAH1 Up R	CTCAGTGTTAGGAACTGCGAACG
5-52 ry 2u-PAH1 Up F	GGGCGGAGCCTATGGAAAAACGCCGTAGAGTCCAAACTCAACAGCCGC
5-53 ry PAH1 Up-Leu2 F	TCTCTTCGTTCGCAGTTCCTAACACTGAGTAACCATTATTTTTTTCCTCAACATAACGAG
5-54 ry Leu2-PAH1 Down R	ATGCACCTTTTTCTTATTTCACTGCAGTTTCTTAAGCAAGGATTTTCTTAACTTCTTCGG
5-55 ry PAH1 Down F	AAACTGCAGTGAAATAAGAAAAAGGTGC
5-56 ry PAH1 Down R	TAGCTGGCAAAATTGGTATTATTCTCTCTC
5-57 ry PAH1 Down - 2u R	TTCTACAAAATGAAGCACAGATGCTTCGTTTAGCTGGCAAAATTGGTATTATTCTCTCTC
5-58 jm 7DLH F	CCAGAAAAACCATCACGACCTGG
5-59 jm 7DLH R	CAAGATCCCCAAGAAATCATCAGCG
5-60 jm ADH2p-solu-trSGD F	TCGTAATACCATGGCAAAGAAGACGAGTTCCAAAGGAAAAGGGAGCAAAGACGACCAATC
5-61 jm ADH2P-solu-trSGD R	TTGGAACTCGTCTTCTTTGCCATGGTATTACGATATAGTTAATAGTTGATAGTTGATTGt
5-62 jm ori-TDH3p F	GGGCGGAGCCTATGGAAAAACGCCGACAGTTTATTCCTGGCATCCAC
5-63 jm TDH3p-G8H R	CAAGGTCAAAATAATGGTCAGGTAGTCCATTTTGTTTGTTTATGTGTGTTTATTCGAAAC
5-64 jm ori-TDH3p R	GGCTCCATTATATTTAGTGGATGCCAGGAATAAACTGTCGGCGTTTTTCCATAGGCTCCG
5-65 jm TDH3p-G8H F	TTAGTTTCGAATAAACACACATAAACAAACAAAATGGACTACCTGACCATTATTTTGACC
5-66 jm Glu1-SPG5t F	GCATTCGATACGCCTCGTAAGAGACTTCGTAAATATTAGGCAAAGACGTTGTTTCATCGC
5-67 jm ADH2P-Glu1 R	GGGTAGGCAGAACGCTACTCATGGTATTACGATATAGTTAATAGTTGATAGTTGATTG
5-68 jm TDH3p-URA3 R	ttacccaattCTCATGTTTGACAGCTTATCATCGGATCACAGTTTATTCCTGGCATCCAC
5-69 jm RsSGD P373T F	CAAGTCACGAAAACCACGGAAAGAAACCAG
5-70 jm RsSGD P373T R	CTGGTTTCTTTCCGTGGTTTTCGTGACTTGATC
5-71 ry ADH2P-ZWF1 F	AATCAACTATCAACTATTAACTATATCGTAATACCATGAGTGAAGGCCCCGTCAAATTCG
5-72 ry ZWF1-SPG5t R	GTAATAGCGCGATGAAACAACGTCTTTGCCTAATTATCCTTCGTATCTTCTGGCTTAGTC
5-73 ry ICL1p-SAM2 F	GCATAACATAACAAAAAGTCAACGAAAAATGTCCAAGAGCAAAACTTTCTTATTTACCTC
5-74 ry SAM2-CPS1t R	AAAATCTTTGACTATTCAATCATTGCGCTTAAAATTCCAATTTCTTTGGTTTTTCCCATG
5-75 jm PCK1P-MsLAMT F	CAAACTCACGCAACTAATTATTCCATAATAAAATAACAACatggccccaacactggacac

5-76 jm MsLAMT-CPS1t R	AAAAAAATCTTTGACTATTCAATCATTGCGCttaattgctcttacgttttagaacaagga
5-77 ry ori-SCS2 F	GGGCGGAGCCTATGGAAAAACGCCGCACGAGCTTGTATGAACAAGCTTTGC
5-78 ry SCS2 UP R	ACTTAGGTTCGCGGAGATTGTAGAATACC
5-79 ry SCS2-Leu2 F	GGTATTCTACAATCTCCGCGAACCTAAGTTAACCATTATTTTTTTCCTCAACATAACGAG
5-80 ry Leu2-SCS2 R	TAGAATACAGCTATATCCTCAATCTCCCTACTTAAGCAAGGATTTTCTTAACTTCTTCGG
5-81 ry SCS2 Down F	TAGGGAGATTGAGGATATAGCTGTATTCTA
6-1 ry SCS2-2uori R	tttTGTTCTACAAAATGAAGCACAGATGCTTCGTTGTGAGTCCCTCGTTCACTATGAGAC
6-2 jm ADH2p-RsSGD F	cataCAATCAACTATCAACTATTAACTATATCGTAATACCATGGACAACACGCAGGCGGA
6-3 jm RsSGD-DockB R	CCAGATCCTCCTCCACCACTCCCTCCCCCTCCAGTCTTCTGCCTCTTAACTAGTTCCACC
6-4 jm RsSGD-DockB F	CGTGAAGAGGCACAGGTGGAACTAGTTAAGAGGCAGAAGACTGGAGGGGGAGGGAGTGGT
6-5 jm PCK1p-NcISYB F	ACTCACGCAACTAATTATTCCATAATAAAATAACAACATGAACTGGTGGAGGGATGGAGC
6-6 jm NcISYB-CPS1t R	CTTTGACTATTCAATCATTGCGCTTAAGAAATAGTAGAGGAAGGAACAATCTTGTAAGCC
6-7 jm MLS1p-NcMLPL F	GTAGTAAAAGCACATAAAAGAATTAAGAAAATGGCTTCCAAGCTTGAAATAGAAATTGAG
6-8 jm NcMLPL-SPG5t R	CTTGGTAATAGCGCGATGAAACAACGTCTTTGCTTAATTTTGACATGTGTGGTTCATGCC
6-9 jm ADH2p-Leu2 F	CGTTTGTTTGCCCCTACGTTTTGCCTTAAGCAAGGATTTTCTTAACTTCTTCGGCGACAG
6-10 jm ADH2p-Leu2 R	TGTCGCCGAAGAAGTTAAGAAAATCCTTGCTTAAGGCAAAACGTAGGGGCAAACAAACGG
6-11 ry SCS2 Up F	CACGAGCTTGTATGAACAAGCTTTGC
6-12 ry SCS2 Down R	GTGAGTCCCTCGTTCACTATGAGAC
6-13 RY ori SNQ2 Up F	GGGCGGAGCCTATGGAAAAACGCCGTCCGCGGAGCTATTTTAAGTTTCCG
6-14 RY SNQ2 Down ori R	tttTGTTCTACAAAATGAAGCACAGATGCTTCGTTTAAAGAAGGGACAGGACAGGTAAGG
6-15 RY ori PTR2 Up F	GGGCGGAGCCTATGGAAAAACGCCGAAGAACAGGAAAAAGGACAACCGTC
6-16 RY PTR2 Down ori R	TTTGTTCTACAAAATGAAGCACAGATGCTTCGTTAAAGAGAAAGTGTGGTCACACCAACC
6-17 RY PTR2-LEU2 F	AAACTCTTATAATGCTCAACCATCCCAGCTAACCATTATTTTTTTCCTCAACATAACGAG
6-18 RY LEU2-PTR2 R	TAAACGCACTAATATTTGGTGGTGGATCTCTTAAGCAAGGATTTTCTTAACTTCTTCGGC
6-19 RY SNQ2 Up LEU2 F	ATCGAAGACCGAAAGCAGTAAAAAAGTGGTAACCATTATTTTTTTCCTCAACATAACGAG
6-20 RY LEU2 SNQ2 Down R	GATACGGGGCTTAGGAAGGAAGATTGTCTTTTAAGCAAGGATTTTCTTAACTTCTTCGGC
6-21 RY SNQ2 Up F	TCCGCGGAGCTATTTTAAGTTTCCG
6-22 RY SNQ2 Down R	TAAAGAAGGGACAGGACAGGTAAGG
6-23 RY PTR2 Up F	AAGAACAGGAAAAAGGACAACCGTC

6-24 RY PTR2 Down R	AAAGAGAAAGTGTGGTCACACCAAC
6-25 jm ICL1p-CpDCS R	CCGTCTTCCTGACTTTTCCCGGCCATTTTTCGTTGACTTTTTGTTATG TTATGCTAAGAG
6-26 jm CpDCS-CPS1t F	TGTAGACATAGGTAACACGCTAAAGTCTGCGTAGGCGCAATGATTGA ATAGTCAAAGATT
6-27 jm ICL1p-MsDCS R	TCCTCCTGGGCACACTTACCGGCCATTTTTCGTTGACTTTTTGTTATG TTATGCTAAGAG
6-28 jm MsDCS-CPS1t F	TGTCGATATCGGAAACACCCTTAAGAGTGCTTAGGCGCAATGATTGA ATAGTCAAAGATT
6-29 jm ADH2p-PcPsiH R	AAAACTAAACAGCACAGCAATCATGGTATTACGATATAGTTAATAGTT GATAGTTGATTG
6-30 jm PcPsiH-SPG5t F	CGTACTGAGCAGGTGAGCCAGTCCGTGTCCGGACCTTAGGCAAAGA CGTTGTTTCATCGC
6-31 jm ICL1p-PcCPR R	AATACATCACTGGAGCTGGAAGCCATTTTTCGTTGACTTTTTGTTATG TTATGCTAAGAG
6-32 jm PcCPR-PRM9t F	AAAGGAGCCGTCTTATGCTTGACGTCTGGTCATAAGACAGAAGACG GGAGACACTAGCAC
6-33 jm CpDCS-PRM9t R	GGTAAAGTTGTGTGCTAGTGTCTCCCGTCTTCTGTCCTACGCAGACT TTAGCGTGTTACC
6-34 jm PCK1p-MsEnolMT4 R	CTTTTTCTTCCACGCTGTGGTTGCATGTTGTTATTTTATTATGGAATA ATTAGTTGCGTG
6-35 jm MsEnolMT4-CPS1t F	GTTTGTACTATTAAAACGTAATGCGGAAGACTAGGCGCAATGATTGA ATAGTCAAAGATT
6-36 jm MsDCS1-PRM9t R	GTAAAGTTGTGTGCTAGTGTCTCCCGTCTTCTGTCCTAAGCACTCTT AAGGGTGTTTCCG
6-37 jm MsDCS2-PRM9t R	TGCTAGTGTCTCCCGTCTTCTGTCCTAGTACAGGGTATTACCAATAT CAATAACAAAACG
6-38 jm ICL1p-MsDCS2 R	TCCTCCTCGGGACTCTTTTCCGCCATTTTTCGTTGACTTTTTGTTATG TTATGCTAAGAG
6-39 jm MsDCS2-CPS1t F	TTTTGTTATTGATATTGGTAATACCCTGTACTAGGCGCAATGATTGAA TAGTCAAAGATT
6-40 ry ATF1 Up-Galp F	CAGTAATGAAGCAAATATTAGAAGAATTCTTTCAAAAATTCTTACTTTT TTTTTGGATGG
6-41 ry CYC1t-ATF1 Down R	AAGCTTCCGAAATTACTTCATGGTAGTGCTCAATAAGTGGCTTCGAG CGTCCCAAAACCT
6-42 ry ATF1 Up-ADH2p F	TACAGTGCAGTAATGAAGCAAATATTAGAAGAATTCGCAAAACGTAG GGGCAAACAAACG
6-43 ry IDP1t-ATF1 Down R	CGAAATTACTTCATGGTAGTGCTCAATAAGTGGGATGGTAATGATCC GAACTTGGGGAAC
6-44 ry ATF1 Up R	GAATTCTTCTAATATTTGCTTCATTACTGCACTG
6-45 ry ATF1 Down F	CCACTTATTGAGCACTACCATGAAGTAAT
6-46 jm PCK1p-CrTDC F	TCACGCAACTAATTATTCCATAATAAAATAACAACATGGGTTCCATTG ATTCTACCAACG
6-47 jm CrTDC-CPS1t R	AAAATCTTTGACTATTCAATCATTGCGCTTAAGCTTCTTTCAACAAAT CGTCAGTCAG
6-48 jm PCK1p-AtIGMT1 R	AGGGTTTCCTGAAATAGATAGCCCATGTTGTTATTTTATTATGGAATA ATTAGTTGCGTG
6-49 jm AtIGMT1-CPS1t F	CTACCACTGTTGGATTATAGAGTTCTGCAAATAGGCGCAATGATTGA ATAGTCAAAGATT
6-50 jm PCK1p-AtIGMT2 R	AACGTCTCCTCAAAAAGATATCCCATGTTGTTATTTTATTATGGAATA ATTAGTTGCGTG
6-51 jm AtIGMT2-CPS1t F	ATACCACTGTTGGATTATTGAGTTTTGTAAGTAGGCGCAATGATTGAA TAGTCAAAGATT

6-52 jm PCK1p-AtIGMT3 R	AGAGTTTCTTCAAAAAGGTAACCCATGTTGTTATTTTATTATGGAATA ATTAGTTGCGTG
6-53 jm AtIGMT3-CPS1t F	GTATCATTGCTGGATCATCGAATTTTGTAAGTAGGCGCAATGATTGA ATAGTCAAAGATT
6-54 jm PCK1p-AtIGMT4 R	AACGTCTCTTCTAGTAGATAACCCATGTTGTTATTTTATTATGGAATA ATTAGTTGCGTG
6-55 jm AtIGMT4-CPS1t F	TTACCATTGCTGGATTATCGAATTTTGTAAGTAGGCGCAATGATTGAA TAGTCAAAGATT
6-56 jm PsiH F	ATGATTGCTGTGCTGTTTAGTTTTG
6-57 jm PsiH R	CTAAGGTCCGGACACGGACTG
6-58 jm pET28A-MsDCS1 F	CAGCAGCGGCCTGGTGCCGCGCGGCAGCCATATGGCCGGTAAGTG TGCCC
6-59 jm MsDCS1-pET-28A R	CGGATCTCAGTGGTGGTGGTGGTGGTGCTCGAGCTAAGCACTCTTA AGGGTGTTTCCG
6-60 jm pET28A-MsEnolMT4 F	CACAGCAGCGGCCTGGTGCCGCGCGGCAGCCATATGCAACCACAG CGTGG
6-61 jm MsEnolMT4-pET28A R	GGATCTCAGTGGTGGTGGTGGTGGTGCTCGAGCTAGTCTTCCGCAT TACG
6-62 jm CYC1t F	ATCCGCTCTAACCGAAAAGGAAG
6-63 ry YCF1 Up F	TTGATCTTGAAAAATAGCACTTTGGAGACG
6-64 ry ori-YCF1 Up F	GGGCGGAGCCTATGGAAAAACGCCGTTGATCTTGAAAAATAGCACT TTGGAGACG
6-65 ry YCF1 Up-Leu2 F	GGTATCGTACTACCGTAAAGAACAAGAAATAACCATTATTTTTTTCCT CAACATAACGAG
6-66 ry YCF1 Up-Leu2 R	TCGTTATGTTGAGGAAAAAAATAATGGTTATTTCTTGTTCTTTACGGT AGTACGATACCC
6-67 ry Leu2-YCF1 Down F	TCGCCGAAGAAGTTAAGAAAATCCTTGCTTAAGACTGTGAAACTAAT AAAAACCTGTCCC
6-68 ry Leu2-YCF1 Down R	TGCGGGACAGGTTTTTATTAGTTTCACAGTCTTAAGCAAGGATTTTCT TAACTTCTTCGG
6-69 ry YCF1 Down-2u R	TTCTACAAAATGAAGCACAGATGCTTCGTTCAATCCTTATTTGTTATG ACACCAGAGTGC
6-70 ry YCF1 Down R	CAATCCTTATTTGTTATGACACCAGAGTGC
6-71 ry YOR1 Up F	TTTTTATTAGTCGCCTTCTTTAGTTGCTGC
6-72 ry ori-YOR1 Up F	GGGCGGAGCCTATGGAAAAACGCCGTTTTTATTAGTCGCCTTCTTTA GTTGCTGC
6-73 ry YOR1 Up-Leu2 F	AAAGAGTAAAGCCGTTGCTATATACGAATTAACCATTATTTTTTTCCT CAACATAACGAG
6-74 ry YOR1 Up-Leu2 R	TCGTTATGTTGAGGAAAAAAATAATGGTTAATTCGTATATAGCAACGG CTTTACTCTTTT
6-75 ry YOR1 Down F	CCGAAGAAGTTAAGAAAATCCTTGCTTAAGTATGTTGCCGATGGTAC AAATTAGTACTAG
6-76 ry Leu2-YOR1 Down R	CTAGTACTAATTTGTACCATCGGCAACATACTTAAGCAAGGATTTTCT TAACTTCTTCGG
6-77 ry YOR1 Down - 2u R	TTCTACAAAATGAAGCACAGATGCTTCGTTTTTTTGAAGCCTTTGATG AAGATTGGAAGC
6-78 ry YOR1 Down R	TTTTTGAAGCCTTTGATGAAGATTGGAAGC
6-79 ry YOR1 Up-ADH2p F	TATTCAAAAAGAGTAAAGCCGTTGCTATATACGAATGCAAAACGTAG GGGCAAACAAACG
6-80 ry IPD1t-YOR1 Down R	TTTCTAGTACTAATTTGTACCATCGGCAACATAGATGGTAATGATCCG AACTTGGGGAAC

6-81 jm CPS1t- YOR1 Down R	TTTCTAGTACTAATTTGTACCATCGGCAACATAATTTGACACTTGATT TGACACTTCTTT
7-1 jm pET28A-AtIGMT4 F	CAGCAGCGGCCTGGTGCCGCGCGGCAGCCATATGGGTTATCTACTA GAAGAGACGTTGTC
7-2 jm pET28A-AtIGMT4 R	TCTCAGTGGTGGTGGTGGTGCTCGAGCTACTTACAAAATTCGAT AATCCAGCAATGG
7-3 jm YOR1 Up R	ATTCGTATATAGCAACGGCTTTACTCTTTTTG
7-4 jm YOR1 Down F	TATGTTGCCGATGGTACAAATTAGTACTAG

Appendix B – Gene Sequences Used in this Study

Gene	Sequence
CrCPR	ATGGACTCTTCTTCCGAGAAGTTGTCTCCCTTTGAATTGATGTCCGCTATTTTGAAGGGTGCTA AATTGGACGGTTCCAATTCTTCTGATTCAGGTGTTGCAGTTTCTCCCGCCGTCATGGCTATGT TGTTGGAAAATAAAGAGTTGGTCATGATTTTGACCACTTCTGTCGCTGTTTTGATCGGTTGTGT TGTTGTTCTGATCTGGAGGCGCTCTTCTGGCTCTGGTAAAAAAGTTGTTGAGCCACCAAAGTT GATTGTTCCAAAATCCGTCGTCGAGCCCGAAGAGATCGACGAGGGCAAGAAAAAGTTCACCA TTTTTTTTGGTACTCAAACTGGCACTGCTGAAGGTTTTGCTAAAGCTCTGGCTGAAGAAGCCA AAGCCAGGTATGAAAAGGCCGTGATTAAGGTCATTGATATCGATGATTATGCCGCCGATGAC GAAGAGTACGAAGAGAAATTTAGAAAGGAGACGTTGGCCTTCTTCATTCTGGCCACTTATGGT GATGGCGAACCAACTGACAATGCTGCTAGGTTTTACAAGTGGTTTGTTGAAGGTAACGATAGG GGCGATTGGTTGAAAAATTTGCAATACGGCGTCTTTGGCCTGGGCAATCGCCAATATGAACAC TTTAATAAGATCGCTAAAGTCGTCGATGAGAAAGTTGCCGAGCAAGGCGGTAAAAGGATTGTC CCATTGGTTTTGGGTGACGATGATCAATGTATAGAAGATGATTTCGCAGCTTGGAGGGAAAAT GTCTGGCCTGAACTGGACAATTTGTTGAGGGATGAAGATGACACTACTGTTTCCACTACCTAC ACTGCTGCTATTCCCGAATATAGGGTCGTCTTCCCAGATAAGTCTGATTCTTTGATCTCCGAA GCCAATGGTCATGCCAATGGCTACGCTAATGGTAATACTGTTTATGATGCTCAACACCCCTGT AGGTCTAATGTTGCTGTCAGGAAAGAGTTACATACTCCAGCCTCTGACAGGTCTTGTACTCAT TTGGATTTCGATATTGCTGGTACTGGTTTGTCCTATGGTACAGGTGATCATGTTGGTGTCTACT GCGATAATTTGTCCGAAACCGTCGAAGAAGCTGAAAGATTGTTGAACCTGCCACCAGAAACTT ACTTTTCCTTGCATGCTGATAAAGAGGATGGTACTCCACTAGCTGGTTCTTCCTTGCCACCAC CATTTCCACCATGTACTTTGAGGACTGCTTTGACTAGATACGCCGATTTATTGAACACCCCAAA AAAGTCTGCTTTGCTGGCTTTAGCTGCTTATGCTTCTGATCCCAATGAAGCCGATAGATTGAA GTATCTGGCTTCCCCAGCCGGTAAAGACGAATATGCTCAATCTTTGGTTGCTAATCAAAGGTC CTTGTTAGAAGTTATGGCTGAGTTTCCATCCGCTAAACCACCCTTGGGTGTCTTCTTCGCTGC TATCGCTCCAAGATTGCAACCAAGATTCTATTCCATTTCTTCTTCTCCAAGGATGGCTCCCTCT CGCATTCACGTTACCTGTGCTTTGGTTTATGAAAAAACTCCAGGCGGTAGGATTCACAAAGGT GTCTGTTCTACCTGGATGAAAAATGCAATTCCCTTGGAAGAATCCCGCGATTGTAGCTGGGCT CCAATTTTTGTTAGGCAATCTAACTTCAAGTTGCCAGCTGATCCAAAAGTTCCCGTCATTATGA TTGGCCCAGGTACTGGATTGGCTCCATTTAGGGGTTTTTTGCAAGAACGCTTGGCCTTGAAAG AAGAAGGTGCTGAATTGGGTACCGCTGTGTTTTTCTTCGGTTGTAGGAACCGCAAGATGGATT ACATTTATGAAGACGAGCTGAACCATTTTCTAGAGATTGGTGCCTTGTCTGAACTGTTGGTCG CTTTTTCCAGAGAGGGCCCCACTAAACAATACGTCCAACACAAAATGGCTGAAAAAGCTTCTG ACATCTGGAGAATGATTTCTGATGGTGCCTATGTTTACGTTTGCGGTGACGCCAAAGGTATGG CTAGAGACGTTCATAGAACTTTGCATACTATTGCGCAGGAGCAAGGTTCCATGGATTCTACTC AGGCTGAGGGCTTTGTCAAGAATTTACAAATGACTGGCAGGTATTTGAGAGATGTTTGGTAA
CrCYB5	ATGGCTTCTGACCAGAAGTTGCACAAGTTCGACGAAGTTTCCAAGCATAACAAGACCAAGGA CTGCTGGTTGATCATCAACGGTAAGGTCTACGACGTTACCCCATTCATGGACGATCATCCAG GTGGTGATGAAGTCTTGTTGTCTGCTACTGGTAAGGATGCTACCAACGATTTCGAGGATGTC GGTCACTCTGATTCCGCTAGGGAGATGATGGACAAGTACTACATTGGTGAGATGGATATGG CTACTGTTCCATTGAAGAGGACCTACATCCCACCACAACAGGCCCAATACAACCCAGACAAG ACCCCCGAATTCGTCATTAAGATCCTGCAATTCCTGGTTCCCTTGTTGATCTTGGGCTTGGC CTTCGCTGTTAGACATTACACTAAGGAAAAGTGA
CrCYPADH	ATGCAGATCATAACTTGCAAGGCTGTGGTGTGCTGGGCGGCCGGAGAGCCACCGGTGGTT GAGGAGATACTGGTAGAACCTCCGAGGTCAGGCGAAGTCAGGATTAAGATTTTGTTTGCTAG TCTTTGCCACACTGATGTCCTCGCCTGCAAGGGCTTCCCAACGCCCATGTTTCCTCGAGTTC

	TGGGACATGAAGGTGTCGGCGTGGTGGAGTGTGTGGGTGAAGGAGTTTCAGAACTGAGAG AGGGAGACGTGGTGATCCCCACATACTTGGGAGAATGCGGAGAATGTGAGAATTGTGAGTC AGGAAGAACGAATCTATGCCGAACTTACCCTTTGCAAGCATTCACAGGCTTAATGCCTGATG GTTCCTCAAGAATGTCTTCCGCCAAAGGAGGGGAAATGTTGTACCAATTCCTTAGCTGCTCC ACTTGGTCTGAGTATACTGTTATTGACGCCAACTATGCCGTGAAGATAGACTCCAGAATACC TCTGCCCCATGCTAGCTTCCTTTCTTGCGGCTTCACCACTGGGTTTGGGGCAACCTGGAAG GAAGCCAAGCTTCAAGAGGGATCCAGCACCGTTGCTGTTCTGGGTCTTGGGGCAGTTGGAC TTGGAGCTGTGGAGGGAGCTCGAGTGCAGGGAGTAACTCAAATAATAGGAATAGACATTAA CGACAACAAACGTGAGAAAGGAGAAGCCTTCGGAATGACTCATTTCATCAACCCCAAAAAAG ATAATAATAAATCCATTTCAGAATTAGTTAAAGAGTTAACAAAAGGACAAGGTGTGGACGTCT GTTTTGAATGCACGGGAGTCCCTGACTTGGTTAATGAAGCTCTTGAATCCACAAAGATCGGA ACAGGAAATATGATAATGCTAGGAGCAGGAACCCAGAAAAGCATGACCATAAACTTCGTTTC ACTATTGGGCTGCAGAACTTTCAAGTATTCTGTTTTCGGCGGGGTTAAGGTCCAATCCGACC TTCCTCTCATTATTCAGAAATGCTTAAATAAGGAAATACAGAAAATTGAGCAGCTTTTAACTCA TCAAGTTCAACTGGAAGACATAAATAGAGCCTTTGAGCTGCTTAAGGAACCTGATTGCGTGA AGGTTCTCATCACATTGTGA
CrG8H	ATGGACTACCTGACCATTATTTTGACCTTGTTGTTTGCTCTAACCTTGTACGAGGCTTTCTCT TACTTGTCCCGTCGCACCAAGAATTTGCCACCAGGTCCATCTCCATTGCCATTCATTGGTTCT TTGCATTTGTTGGGCGACCAACCCCATAAAAGCTTGGCTAAATTGTCCAAGAAGCACGGTCC AATCATGTCCTTGAAGTTGGGTCAGATCACTACTATCGTCATCTCCTCCTCTACCATGGCTAA AGAAGTCTTGCAAAAACAGGACCTGGCCTTTTCTTCTAGGTCTGTTCCAAACGCCCTGCATG CTCACAACCAATTCAAATTTTCTGTCGTCTGGTTGCCCGTTGCTTCCAGATGGAGATCTTTGC GCAAGGTCTTGAATTCCAACATTTTTTCCGGCAACCGCTTGGACGCTAACCAGCATTTGAGG ACTCGCAAAGTTCAGGAATTGATCGCTTACTGTAGGAAGAATTCCCAATCTGGTGAAGCTGT CGATGTCGGTAGAGCTGCTTTTAGGACTTCCCTAAACCTGCTGTCTAACTTGATCTTTTCCAA GGACTTGACTGACCCCTATTCTGACTCTGCCAAGGAGTTCAAGGATCTAGTCTGGAACATTA TGGTCGAAGCTGGTAAACCAAACTTGGTCGACTTTTTTCCCTTGCTGGAAAAGGTCGATCCA CAAGGCATCAGGCATAGAATGACCATCCATTTTGGCGAGGTTTTGAAGCTATTTGGCGGCTT GGTCAACGAAAGATTGGAACAAAGGAGGTCTAAGGGTGAAAAGAACGACGTCTTGGACGTT CTGTTGACAACCTCCCAAGAGTCTCCAGAAGAAATTGATAGGACCCATATCGAAAGAATGTG TTTGGACCTGTTCGTTGCTGGTACCGATACTACCTCCTCAACTTTGGAATGGGCCATGTCTG AGATGTTGAAGAACCCAGATAAGATGAAGAAGACTCAGGATGAATTGGCCCAAGTTATCGGC AGGGGTAAGACTATCGAGGAGTCCGACATTAATAGACTGCCATATTTGAGGTGCGTCATGAA GGAAACATTGCGCATTCATCCACCAGTCCCATTTTTGATCCCAAGGAAGGTCGAGCAATCTG TTGAGGTTTGCGGTTATAACGTTCCAAAGGGTTCTCAAGTCTTAGTCAATGCTTGGGCTATTG GTAGGGATGAAACTGTCTGGGATGACGCCTTGGCTTTCAAACCAGAAAGATTCATGGAATCT GAACTGGACATTAGAGGTAGGGACTTTGAACTGATTCCCTTTGGTGCTGGCAGACGCATTTG TCCAGGTTTGCCATTGGCTTTGAGAACAGTTCCATTGATGTTGGGTTCCTTGCTGAACAGCT TTAATTGGAAATTGGAAGGCGGTATGGCCCCCAAGGATTTGGATATGGAGGAAAAGTTCGG CATTACTTTGCAAAAGGCTCATCCATTGAGAGCTGTTCCATCTACTTTGTAA
CrGOR	ATGACTAAAACTAATTCTCCAGCCCCATCTGTCATTACTTGCAAGGCTGCTGTCGTTTGGAAA TCCGGTGAACCACCAAAGGTCGAAGAGATCCAAGTTGATCCACCCAAGGCTTCTGAAGTTC GCATTAAGATGTTGTGTGCTTCCTTGTGCCACACCGATTTCTTGGCTTGTAATGGTCTGCCA GTTCCATTGTTTCCCAGAATTCCAGGTCACGAAGGTGTTGGTATGATCGAATCTGTCGGTGA AAACGTCACCAACTTGAAGGAAGGTGACATTGTCATGCCATTGTACTTGGGTGAGTGTGGCG AATGCTTGAATTGCAAGTCCGGCAGGACTAACTTGTGTCATAAGTATCCGTTGGGTTTTTCTG GCCTGTTGTTGGATGGCACTTCCAGGATGAGCATTGGCGAACAAAAAGTCTACCACCACTTC TCTTGTTCCACCTGGTCTGAATACATTGTTATTGAGGCCGCCTACGCAGTTAAAGTTGACCC AAGGGTTAGCTTGCCACATGCTTCTTTCCTGTGTTGCGGTTTTACTACTGGCTTTGGCGCCA CTTGGAGAGATGTTAATGTTGTCAAAGGCTCTACTGTCGCTGTTTTGGGTTTAGGTGCCGTC GGTTTGGGTGCTGTTCAAGGCGCTAAATCTCAAGGTGCCTCCAGGATCATTGGTTTAGACAT TAACGATAAGAAGAGGGAGAAAGGCGAAGCTTTCGGCATGACCGAATTCATCAACCCCAAG GGCTCCAATAAGTCCATCTCCGAATTGATCAACGAAGCTACTGGTGGTCTAGGTTTGGACTA CGTTTATGAATGCACTGGTGTCCCAGCTCTGTTGAACGAAGCCATTGAGTCCTCTAAAGTTG GTCTGGGTACTGCCGTCTTGATTGGTGCTGGTCTAGAAACCTCTGGTGAAATCAAATTCATT CCCCTGTTGTGCGGCAGAACTGTTAAAGGTTCCATTTACGGTGGTGTTAGGCCAAAGTCCGA CTTGCCAACTCTGATTGAGAAGTGCATTAACAAGGAGATTCCAATGGACGAGCTGATGACCC ATGAGGTGTCTCTGTCCGAGATCAACAAGGGTTTCGAGTACTTGAAGCACCCAGACTGTGTC AAAGTTGTTATTAAGTTCTAA
CrISY	ATGTCCTGGTGGTGGAAAAGGTCTATTGGTGCTGGCAAAAACTTGCCAAACCAAAACAAGGA AAACGGTGTCTGCAAGTCTTACAAATCTGTCGCCTTGGTCGTCGGTGTTACTGGTATTGTTG

	GTTCTTCTCTGGCTGAGGTTTTGAAGTTGCCAGATACTCCAGGTGGTCCATGGAAAGTTTAT GGTGTTGCTAGAAGACCATGTCCAGTCTGGTTGGCTAAGAAGCCAGTCGAGTACATCCAGT GTGACGTCTCCAATAACCAAGAAACCATTTCTAAGCTGTCTCCCCTGAAAGACATCACTCAC ATCTTCTATGTCTCCTGGATTGGCTCTGAGGATTGCCAGACTAATGCCACCATGTTCAAGAA CATCTTGAACTCCGTTATCCCAAATGCTTCCAACTTGCAGCACGTCTGCCTACAAACCGGCA TTAAGCATTACTTCGGCATTTTCGAAGAGGGTTCCAAAGTCGTTCCACATGATTCCCCCTTTA CCGAAGATTTGCCACGCTTGAACGTCCCAAACTTTTATCACGACCTGGAAGACATTTTGTAC GAGGAGACAGGCAAAAATAACCTAACCTGGTCCGTTCACAGGCCAGCTTTGGTTTTCGGTTT TTCCCCATGCTCCATGATGAATATCGTCTCTACTCTGTGCGTCTACGCTACTATTTGCAAGCA TGAGAACAAGGCTCTGGTTTACCCAGGTTCCAAGAATTCCTGGAATTGCTATGCTGATGCTG TCGATGCTGACTTGGTTGCTGAGCATGAAATTTGGGCTGCTGTTGATCCAAAGGCCAAAAAC CAGGTTCTGAATTGCAACAACGGCGACGTCTTCAAATGGAAACATATCTGGAAGAAGCTGGC TGAAGAGTTTGGTATCGAGATGGTCGGTTATGTTGAAGGCAAAGAACAGGTCAGCCTGGCC GAATTGATGAAAGATAAGGATCAAGTCTGGGACGAAATCGTCAAGAAAAACAACCTGGTGCC AACTAAGTTGAAGGAGATTGCCGCCTTCTGGTTTGCCGATATCGCCTTTTGCTCTGAAAACTT GATCTCTTCCATGAACAAGTCCAAGGAGCTGGGTTTCCTAGGCTTCAGGAACTCTATGAAGT CTTTCGTCTCCTGTATCGACAAGATGAGAGACTACAGATTCATTCCATAA
NmMLPL	ATGGCGTCGAAACTGGAGATCGAAATCGAACTTAAATCGGACGTGGAAAAAATGTGGAAACA TTTCAAGGAGTTTACAAAACTGTTCCCGAAGGCTTGCCCGCATTTGTATGAAAAGATTGATGT GATTGAGGGGGATGGCATCTCGGTGGGGACTATTTTCGTGTCAACGTTGAAGCCTACAGAG TTAAATCCAGTAGTGATGGTCACGAAAGAAAAGATTGATTTTTTAGATGACGAAAATAAAATG TTACGCTATTCTTACATGGAGGGTGAGATTTTAAAGAACTACAAGAACCTTCGTGGGACGGT ACACATGTCCAGCTCGAAGTCCGGTGGAACTATCTTCAAGTACTCGGGTGAATTTGAAAAGG CGAACGAACAGGTCCCCGACCCCGTATTCTTTAAGGACTTCATGGTCATTGTCTTTCAAGGG CTTGACGACTACATCTTGAAGGGTATGAATCACACTTGTCAAAATTAG
CrIO	ATGGCTACCATTACGTTTGATTCCTTGAATCCAGTTACCGTCGCCATTTCCGCCGGTTTCTTG CTGCTGCTGATTATTTTCGTTAAGTCTAGGACCGGTTCTTCCAAAAGAAAACCACCAGGCCC ACCAGGTTGGCCAATCTTCGGTAACATGTTTGATTTGGGTGATCTGCCACATCAAACACTGT ACAAATTGAAGTCCAAGTACGGTCCCATTGTCTGGTTGCAACTGGGTTCCATCAATACCATG GTTGTTCAGAATGCTGTTTCCGCTGCTGAGTTGTTCAAGAAGCACGACGTTCCATTTTGTGAT CGCAAAGTTCCCGACACTTTGACCGCGTTCAATTTTAACCAGGGTTCCCTGGGCATGAACAC TTATGGTGGTCATTGGAGAGTTTTGAGGAGATTGTGTTCCATGGAGTTCCTGGTCAACAAGC GCATGAATGAGACTACTGATCTGAGGAGACGTATCGAAGATAACATGGTCAGGTGGATCGA AGAAGATTCTTTGGCTTCTAAAGCCCAAGGTGGTACCGGTGCTGTCCAGTTGTCAAGGTTCT TGTTTTTGATGGCTTTTAACTTGGTCGGCAACTTGATGTTGTCCAGGGACTTGATGGATAACA AGGATCCCGAAGGTCGCGAGTTTTTCGACTGTATGAACGAAATTTTGGAGTTGGCCGGTACC CCCAATATTGCCGACTTCTTGCCATTGCTGAAGAAGTTGGACCCATTGGGTATGAAAAAGAG GATGGTTGACAACATGTCCAGGACCATGAAGATCTCCTCCAAATTCGTGCAGGAAAGACTAG ACAACAGAAAGGCCGGTAAGATCAACGAGAAGAAAGATTTCCTGGACGTTATGCTGGAATAC CAAGGTGACGGTAAGGACGGTCCAGATAAGTTCACCGAGCAGCATGTCAATATTGTCATCAT GGAAATGTTCTTCGCCGGTTCCGAAACTACTTCCATCTCTATTGAGTGGGGCTTTACCGAGT TGTTGAGGAACCCACATGCTTTCAAAAAGGTTAGGGAGGAAATCGATAGAGTGGTTGGTGTC AATAGGATGGTCGAGGAATCTGACATGGAGAACTTGCCCTATTTGCAAGCCGTTGTTAAGGA AACTTTGAGACTGCACCCAGCTTTGCCAATGTTGCTGCCAAGGAATACTATGGAAGACACTG AGTACATGGGTTACTTGATCCCAAAAGGCACTCAAGTCTTTGTCAACGCTTGGGCTATTGGT AGGGACCCCGAATATTGGCAGGATCCATTGTCATTCAAACCCGAAAGGTTCATTAATTCCTC TGTCGAATACAAGGGTCAGCACTTCGAACTGATTCCATTTGGCTCCGGTAGGAGGATTTGCG TTGGTTTTCCATTGGCTCATAGAGTCGTTCATTTGACTTTGGCTACTTTGGTCCAAGCCTTTG ATTGGGACCTGGGTGCTGGTGTTAAACCACAAGATATCGACTTGGAGGAAAGATTGGGTTTG ACATTGCGCAAAAAGAACCCCTTGAACGTCATCCCAAAGAAGAGGGTCCACATCTGA
Cr7DLGT	ATGGGTTCACAAGAGACTAACTTGCCACCACACGTTTTGATTTTCCCCTTGCCAATCCAAGG TCACGTTAATTCCATGTTGAGATTGGCCGAATTGCTGTGTTTGGCCGAGTTGGATATCACCTT CATCGTTAGCGAATTCTCCCACTCTAGGCTGATTAAGCATACTAATGTTGCTTCCAGGTTCGC TAGGTACCCAGGTTTTCAGTTCCAACCAATTTCTGATGGCTTGCCAGACGATCATCCAAGGG CTGGCGAAAGGGTTATGGATATCTTGCCATCTACAAAGAATGTCACCGGCCCATTGTTCAAA CAGATGATGGTTGAGAATAAGTGCTTCTCCTCTGCTACCAGGAGGCCAATTACTTGCATCAT TGCTGACGGTGTTTTGTCTTTTGCTGGCGATTTTGCCCAAGAAAAAGGCATCCCACTGATTTA TTTCAGGACCGTCTCTGCTTGCTCCTTTTGGGCTTGTTTTTGCATGCCAGAATTGATCGAGTC CGGAGACATTCCAATCAAAGGTAACGGTATGGATTTGATCGTTAAGTCCGTCCCAGGTATGG AGACGTTCTTGAGAAGAAGAGATCTGCCAGGTTTCTGCAGAGTCAACGATATTAATGAGCCC AAGCTGCAAATTTTGAAAACCGAAACCAGGCAGACTACCAGAGCTCAAGCTGCCATCCTGAA

	CACCTTCGAAGATTTAGAAGGTCCAATTCTCTCTCAAATCCGCAAACACATGCCAAGGTTGTT CACTATCGGTCCCTCCCATTCTCACCTGACCTCTAGGTTGGAAACTAAGAATATTAAGACCCT AATCTCTTCAGGCTCTTTCTGGGAGGAAGACAGGTCTTGTGTTGACTGGTTGGATGCTCAAC CACCACGTTCTGTCTTGTATGTCTCCTTTGGCTCCATTACTGTTGTCACCAGGGATCAGTTGT TGGAATTCTGGTATGGCTTGGTTAACTCTGGTCAACGCTTTTTGTGGGTCATGAGGCCAGAT TCTATTATGGGCAAGGATGGTCAGTCTCAAATTCCAGCTGATTTGGAAGAGGGTACCAAAGC TAGAGGTTATATGGTCGGTTGGGCTCCACAAGAAGAAGTCCTGAATCATCCAGCCATTGGTG GTTTTTTGACTCATTCCGGATGGAATTCAACTCTGGAGTCTATTGTCGCTGGCGTCCCAATG ATCTGTTGGCCTTACTTCGCTGACCAAATGATTAACTCTCGTTTTGTGTCCGAGATCTGGAAA ATCGGTTTGGACATGAAGGACACTTGTGACCGCGAGACTATTGTCAAGATGGTGAGGGAGT TGATGGAAATTAGGAAGGACGAGTTTTTACAACGTGCTGATCATATGGCCAAGTTGGCCAAG GAAGCTGTTTCCGAAGGTGGTTCCTCTTATAGCAACTTGGATGGCCTGGTTGATTACATTAA GTCCTTGATTATCTGA
Cr7DLH	ATGGAACTGAACTTTAAGTCTATCATCTTTCTGGTCTTCGTTTCCTTGACCCTGTACTGGGTC TACAGGATTTTGGATTGGGTCTGGTTCAAGCCCAAGAAGTTGGAAAAGTGTTTGAGAGAGCA AGGTTTCAAAGGCAACCCATATAGGTTGTTCTTGGGTGATCAGTACGACTCTGGTAAGTTGA TCAGGCAGGCCTTGACTAAGCCAATCGGTGTGGAAGAAGATGTTAAGAAGAGAATTGTTCCA CACATTTTGAAGACCGTTGGCACCCACGGTAAGAAATCTTTCATGTGGGTTGGCAGGATCCC AAGGGTCAACATCACCGATCCCGAACTGATTAAGGAGGTCTTGACCAAATACTATAAGTTCC AGAAAAACCATCACGACCTGGACCCCATTACTAAGCTGTTGCTGACTGGTATCGGTTCCTTG GAAGGTGATCCATGGGCTAAAAGGCGCAAGATTATTAACGCTGCCTTCCATTTCGAAAAGTT GAAGTTGATGTTGCCAGCCTTCTACCTGTCTTGTAGAGACATGGTTACCAAGTGGGACAACA AGGTTCCTGAAGGTGGTTCTGCTGAAGTCGATGTTTGGCATGATATCGAAACCTTAACCGGT GACGTTATTTCTAGGACCTTGTTCGGTTCCAATTTCGAAGAAGGTAGGCGCATTTTCGAGCT GATGAAAGAGTTGACAGCTTTGACCATTGATGTCATCAGGTCTGTCTATATTCCAGGCCAGA GATTCTTGCCCACCAAGAGAAACAACAGGATGCGCGCTATCGACAAGGAAGTCAGAGTCAG GATTACTGAGATCATTAACAAAAAGATGAAGGTCATGAAGTCTGGCGAGGCCGCTTCCGCC GCTGATGATTTCTTGGGGATCTTGTTGGAATGTAACTTGAATGAAATCAAAGAGCAGGGCAA CAACAAGTCCGCCGGTATGACTATCGGCGAGATTATTGGCGAGTGTAAGTTGTTCTACTTCG CCGGTCAAGATACTACTTCCACTTTGTTGGTTTGGACTATGGTTCTGTTGTCTCGCTTTCCAG AATGGCAGACCAGAGCTAGAGAAGAAGTCTTTCAAGTCTTCGGCAACAAAACCCCAGACTAC GATGGCATTTCCCACCTAAAGGTCATCACCATGATTCTGTATGAAGTGTTGAGGTTGTATACT CCAGTTGCTGAGTTGACTAAAGTCGCCCATGAAGCTACTCAGCTGGGTAAGTACTTCATTCC AGCTGGCGTTCAACTGATGATGCCACAAATTTTGCTACATCATGATCCAGAAATCTGGGGTG AGGACGTCATGGAATTCAAACCAGAACGCTTCGCAGAAGGTGTTTTGAAGGCTACTAAGTCT CAGGGTTCCTTTTTTCCATTTTCCTTGGGCCCCAGGATGTGCATTGGTCAAAATTTCGCCTTG TTAGAAGCTAAGATGGCCATGTCCTTGATTTTGAGGAGGTTCTCTTTTGAATTGTCCCCCTCC TATGTCCATGCCCCCTTCACTTTGATTACCATGCAACCCCAATACGGTGCTCATTTAATTTTG CATAAGTTGTGA
CrLAMT	ATGGTTGCTACTATCGATTCTATTGAAATGCCAGCTTTGCCAACTGCCGTTGAAGCCCATCCT ATGAAAGGCGGCGATGATTCTCACTCTTACTCTCAGAACTCCTGCTACCAAAAGGGTGTTAT CGACGCTGCCAAGGCTGTTATTGTCGAAGCCGTCAATGAAAAGCTGGATTTGGAGAATAACC CCATCTTCGACCCAATTAAGCCATTCAGGATCGCTGACTTTGGCTGTTCTACCGGTCCAAAC ACTTTCCATGCCATGCAAAACATTGTTGAGTCCGTCGAGACTAAGTACAAGTCTTTGCAAAAG ACCCCCGAATTCCACGTCTTCTTCAACGACCACGTCAACAACGACTTCAACGTTTTGTTTAGA TCCTTGCCCCCCAACCGCGAATTTTTCGCTGCTGGTGTTCCAGGCTCTTTCTACACTAGGGT TTTCCCAAAGAATTCTATCCACTTCGCCCATTGTTCCTACGCCTTGCATTGGTTGTCCAAGGT TCCCAAGGAAATTCAGGACAAGAACTCCCTGGCTTACAACAAAGGTAGGATTCATTATACTG GCACCGAGAAGCACGTCGTCAAAGCTTACTTCGGCCAGTTCCAAAGAGACTTTGAAGGCTT CTTGAAGGCCAGAGCTCAGGAAATTGTTGTTGGCGGTTTGATGGTCATTCAAATTCCAGGTT TGCCATCTGGTGAGGTCCTGTTTTCTAGGACTGGTGCTGGTCTGCTGCACTTTTTGTTGGGT ACTTCCTTGATGGAGTTGGTTAATAAGGGCATCATCAACGAGGAATCCGTTGACTCCTTCAA CTTGCCACAGTATCACCCCTCCGTGGAAGATTTGGAAATGGTCATCGAAATGAACGATTGCT TCACCATCGAAAGGGTTGGCACCCTGCCACATCCAATGAAGAATTTGCCATTCGACGTTCAG AGGACATCCTTGCAAGTCAGAGCTATTATGGAGTGTATCCTGACCGAGCACTTTGGCGAGAA CATTCTAGACCCCCTGTTTGAGATCTACACAAAGAACTTGCAGGAGAACTTCCACGTTTTCG ACAAGGAGATTAGGAAGGACGCCGACCTATACTTGGTCTTGAAGAGGAAGGGTAATTGA
CrSLS	ATGGAAATGGATATGGATACTATCAGAAAGGCTATTGCTGCAACTATTTTTGCTTTGGTCATG GCTTGGGCCTGGAGAGTTTTGGATTGGGCTTGGTTTACTCCAAAAAGGATCGAAAAACGTTT GAGGCAGCAAGGTTTTAGAGGTAACCCATATAGATTCCTGGTCGGTGATGTCAAGGAATCTG GTAAGATGCACCAAGAAGCCTTGTCTAAACCAATGGAGTTCAACAATGACATTGTTCCCCGC

	TTGATGCCCCATATCAATCACACTATTAACACCTACGGTCGTAATTCCTTCACTTGGATGGGT AGAATTCCAAGAATCCATGTCATGGAGCCAGAACTGATCAAGGAGGTCTTGACTCATTCCTC CAAATACCAGAAGAATTTCGATGTTCACAACCCATTGGTCAAGTTTTTGCTGACTGGTGTCG GTTCTTTTGAAGGTGCTAAGTGGTCCAAGCATAGGAGGATTATCTCTCCAGCTTTCACCTTG GAAAAATTGAAGTCTATGTTGCCAGCCTTCGCCATTTGTTATCACGACATGCTAACTAAGTGG GAGAAGATCGCTGAAAAGCAAGGCTCTCATGAAGTCGATATTTTCCCAACTTTTGACGTTCT GACTTCCGACGTTATTTCCAAAGTTGCTTTCGGTTCCACCTACGAAGAAGGCGGTAAGATCT TCAGGTTGTTGAAAGAATTGATGGATCTGACCATTGATTGCATGAGGGATGTCTATATTCCAG GTTGGTCTTACTTGCCCACTAAGCGCAACAAGCGCATGAAAGAGATCAATAAGGAGATTACC GACATGTTGAGGTTCATCATCAACAAGAGGATGAAGGCTTTGAAGGCCGGTGAACCAGGTG AAGATGACTTGTTGGGTGTTTTGTTGGAATCCAATATTCAGGAAATTCAGAAACAAGGCAATA AGAAGGACGGCGGCATGTCCATCAACGACGTCATCGAAGAGTGCAAGTTGTTCTATTTCGCT GGCCAAGAAACTACTGGTGTGTTGTTGACTTGGACTACTATTTTGTTGAGCAAGCATCCAGA ATGGCAAGAAAGGGCTAGGGAAGAAGTTCTGCAAGCCTTTGGTAAGAATAAACCAGAGTTC GAAAGGTTGAACCACTTGAAGTACGTCTCCATGATTTTGTACGAGGTCCTACGCTTGTATCC ACCAGTGATTGACTTGACTAAGATCGTCCATAAAGACACTAAACTGGGTTCTTACACTATTCC AGCTGGCACCCAAGTTATGCTGCCCACTGTTATGTTGCATAGAGAAAAGTCCATTTGGGGCG AAGATGCTATGGAATTCAATCCAATGCGCTTTGTCGATGGCGTTGCCAATGCCACGAAGAAT AACGTTACTTATTTGCCATTTTCTTGGGGTCCAAGAGTCTGTTTGGGTCAAAACTTTGCCTTA TTGCAAGCCAAGCTGGGTTTGGCCATGATTCTACAGAGATTTAAGTTTGATGTCGCTCCCTC CTACGTTCATGCTCCATTCACCATTTTGACTGTTCAGCCACAATTCGGTTCTCACGTCATCTA TAAGAAGTTGGAAAGTTA
CrSTR	ATGGCTAATTTCTCTGAATCTAAGTCTATGATGGCCGTGTTCTTTATGTTCTTCCTGCTGTTG CTGTCCTCCTCCAGTTCCTCCTCATCCTCTTCCCCAATCTTGAAGAAAATCTTCATTGAGTCT CCATCTTACGCTCCCAACGCTTTCACCTTCGACTCCACCGATAAGGGTTTCTACACTTCTGTT CAAGATGGCAGGGTTATCAAATACGAAGGTCCTAACTCCGGCTTCACTGACTTTGCTTACGC TTCTCCATTCTGGAACAAGGCTTTTTGCGAAAACTCCACTGACCCCGAAAAAAGACCATTGT GCGGTAGGACCTACGATATCTCCTACGACTACAAAAATTCCCAGATGTACATTGTCGACGGT CATTACCATTTGTGCGTTGTCGGTAAGGAAGGTGGTTATGCCACTCAACTAGCTACCTCCGT TCAAGGTGTTCCTTTCAAGTGGTTGTATGCTGTCACCGTTGATCAGAGAACTGGCATTGTCT ACTTTACCGACGTTTCATCTATTCATGACGACTCTCCCGAAGGTGTTGAAGAGATCATGAACA CTTCCGATAGGACTGGTAGGCTGATGAAGTACGATCCCTCTACTAAAGAAACCACTCTGTTG TTGAAGGAACTGCACGTTCCAGGTGGTGCTGAAATTTCTGCCGATGGTTCTTTCGTTGTTGT TGCTGAATTCCTGTCCAATAGGATCGTTAAGTACTGGTTGGAAGGTCCAAAGAAGGGCTCTG CCGAATTCTTGGTCACCATTCCAAATCCAGGTAATATCAAGAGGAATTCTGACGGCCATTTCT GGGTTTCTTCTTCTGAGGAATTGGACGGTGGTCAACATGGTAGAGTCGTGTCTAGGGGTATC AAATTCGATGGTTTTGGTAACATCTTGCAGGTCATTCCACTACCACCACCATATGAAGGCGA GCATTTCGAGCAGATCCAAGAACATGATGGTCTGTTGTACATCGGTTCCCTGTTCCATTCTTC TGTTGGCATCTTGGTCTACGACGACCATGATAACAAGGGTAACTCCTACGTTTCTTCATAA
Lj7DLH	ATGATGATGAGCTATAACTTAATCGGTGGCTCATTAATCTTCGGGGTCATAACTTATTGGGTC TACTCATTCTTGAATTGGATTTGGTTCCGTCCCAAGAAACTAGAGAAATGTCTGAGAGAGCA GGGTTTTGGAGGCAACGCGTACAGACTATTTCTGGGGGACCAGCAGGAGAGCAAAGTAATG ATAAGAGACGCAATGTCCCGTCCTATTACTTTGTCTGATGACATCAAACAGAGGGTCATTCCT CATGTCCTAAAGACCATGAACAACCATGGCAAGAACTCCTTTATGTGGGTGGGGAGAATGCC TCGTTTACATATCACCGAACCGGAATTGATACGTGACGTGCTAACGAAATACTACAAATTCCA AAAGAACCATCACAGTTTAGACCCAATCACCAAATATCTGTTATCTGGAATTGGTTCTTTGGA GGGAGAACCGTGGGCCCAGAGACGTAGAGTGATTAACAGCGCTTTCCACTTTGAAAAATTG AAACTAATGCTGCCAGCTTTTTACTTGTCCTGCTTGGACATGGTCAATAAGTGGGAGAAAGT AGTCTCCAGTAAGGGAGGGTCTGTAGAGGTGGAAGTGCATCACGACCTAGAGACGCTTACC GGCGACGTAATTTCTCGTACTCTGTTTGGGAGTAATTTCGAGGAGGGGAAAAAGATCTTTGA ATTGATGAAGGAGTTGACGGTCCTAACGATCCAGGTCATCCAAAGTGTGTACATCCCTGGCT GGAGATTCATGCCGACTAAACGTAACAATCGTATTAAAAAGATAGATAAGGATGTTAGAGTGT CCATTACTGAAATCATTAACAACAAAATGAAAGCTATGAAAGCGGGCGAGAGCAGCTCATCC GATTTCCTTGGAATCTTGCTAGAATGCAATATGACAGAGATAGAACAAACGAAAAATAAGAAC GCGGGGCTTAGCATTGAGGAGATAATTGGTGAATGCAAATTGTTCTATTTCGCGGGGCAGG ATACTACTTCCACGCTTCTATGCTGGACGATGGTAATACTATCCCGTTTTCCCGACTGGCAG GCGAGGGCAAGGGAGGAGGTCCTACAGGTTTTTGGAGATGGAAAGCCCGATTATGATGGTA TCAATAGGCTAAAGACAGTGACCATGATATTGTATGAAGTTCTAAGGCTGTATCCACCAGTG GTCGAACTTACCAAAGTCGCTCACGAAGACACCAAACTAGGTGACTTGACGATCCCTGCGG GGGTACAGGTTATGCTGCCGACGATTCTGCTGCACCATAATCCCGATATCTGGGGAGAGGA CGTGGACGAATTTAAGCCGGAACGTTTCGCGCAAGGGGTCTTGAAGGCCACCAAGTCCCAA

	GGTTCCTTTTTTCCGTTTTCACTAGGCCCAAGAATGTGTATTGGTCAAAATTTCGCTCTGCTG GAAGCCAAGATGGCTCTGGCTCTGATACTACCTCGTTTCTCCTTTGAACTAAGTCCCAGTTA CGTCCATGCGCCGTATACTCTGATAACAATGCAACCTCAATTTGGGGCACATCTTATTTTACA TAAAATATAA
Rs7DLH	ATGGAAGTCTCCTTCAAAAGCGTTACTGTGCTGGGTTTCGTCGGTTTAGCATTGTATTGGGT TTATAGGGTCTTAGATTGGATTTGGTTCCGTCCGAAAAAGTTAGAAAAATGCTTAAGAGAGCA GGGTTTTAAAGGAAATCCATATCGTCTTTTCTTAGGTGATCAATACGAGTCTGGCAAACTTAT AAAGGAGGCTATGAGCAAACCGATCGGGGTGGAAGAGGATGTCAAAAAACGTATTGTGCCG CACATCTTGAAGACGGTTGAAACTCACGGAAAGAACAGCTTTATGTGGGTTGGAAGAATTCC AAGAGTGCAGATTACCGATCCCGAGCTAATTAAAGAAGTACTTACCAAGTATTATAAGTTCCA GAAAAATCATCATGACTTGGACCCGATTACCAAGTTCCTTCTGACTGGTATAGGAAGCCTAG AAGGGGAAACATGGGCTAAGCGTAGGAAGATAATAAATGCAGCGTTCCACTTTGAGAAACTG AAGTTAATGCTACCCGCGTTCTACCTTAGCTGCCGTGATATGGTCGCTAAGTGGGACAAAAA GGTCCCCGAAGGCGGATCAGCTGAAGTCGATGTATGGCACGACATAGAGACGCTGACTGG GGATGTGATATCCAGGACGTTGTTTGGATCTAACTATGGGGAGGGAAGGAGGATCTTTGAG CTTATGAAAGAGTTGACTGCGCTTACCATAGACGTAATAAGGAGTGTATATATACCAGGCCA TAGGTTCTTGCCCACCAAGCGTAATAATAGAATGCGTGCAATTGATAAAGAGGTCAGAGTGA GGATCACTGAAATTATAAATAAAAAGACCAAGATCATGAAAGCTGGTGAAGCTGCCGCAGCT GATGACTTTCTGGGGATTTTACTAGAATGTAATCTGAACGAGATCCGTGAGCAAGGTCACAA CAAGACCGCTGGAATGACTATTGAGGAAATCATCGGGGAGTGTAAACTTTTTTATTTTGCGG GCCAAGATACGACGAGTACGTTGTTGGTCTGGACAATGGTCTTACTGTCTCGTTTCCCAGAA TGGCAAACAAGAGCGCGTGAAGAGGTCTTCCAAGTGTTCGGTAACAAAACACCTGATTATGA CGGGATCTCTCACTTAAAAGTGATTACCATGATCTTATACGAGGTTTTGAGGCTGTACACCC CTGTGGCCGAGCTAACTAAAGTGGCGCACGAAGATACGCAACTGGGAAAATACCTTATACCT GCCGGCGTTCAACTTATGATGCCTCAAGTGCTACTTCACCACGACCCTGAAATTTGGGGGG AAGATGTGATGGAGTTTAAACCAGAGCGTTTTGCTGAAGGTGTGTTAAAAGCAACGAAATCA CAAGGCAGCTTCTTTCCTTTCAGCTTGGGGCCTCGTATGTGTATTGGGCAGAACTTTGCATT GCTGGAAGCGAAAATGGCAATGACCTTGATTTTAAGGAGGTTCAGTTTTGAGTTGAGCCTTT CTTACGTTCACGCCCCTTTCACCTTAATAACCATGCAGCCTCAGTATGGGGCTCACCTGATT TTACATAAGCTGTAG
Ca565	ATGGAGATACAAATGGATGTGCTATACAAGTCCATAGCTGCAAGTGTGGCGGTAGTTTTCTT GGTGTACGCTTGGAAAATGTTAAATTGGGCTTATCTAACCCCGAAAAGAATTGAGAAGTGTC TTAGGAAGCAGGGATTCAAAGGGAACTCATATAGACTGTTAGTTGGGGATTTAAAAGAAAGT TCTATGATGTTAAAGGAGACCATGAGCAAGCCGATCAACGTCTCCGAGGATATCGTTCAAAG GGTAATGCCGCACGTAATTAAGACAATCGACACATATGGCAAGAACAGCTTTACATGGATAG GACGTATGCCTAGGGTCCACATAATGGAGCCCGATCTGATTAAAGATATTTTAGCAAACCAC AACGACTTTATGAAAAACCACCACGCGTATAATCCGCTAACGAAGTTCCTACTAACAGGTATT GGATCTTTGGAGGGCGATAAATGGGCGAAACATAGGCGTATAATCTCTCCCTCATTTCACCT GGAGAAGTTAAAAACAATGCTTCCAGCCTTCTATGTCTCCTATGACGATCTTCTTACAAAGTG GGAACAACAATGTAGTTCAAAGGGGAGTGTTGAGATTGATCTATTTCCCACCTTTGATACACT TACATCTGATGTTATATCTAGAGTGGCTTTCGGGTCTTCATACGGCGAAGGGGGCCGTATCT TTATCCTTTTAAAAGAACTGATGGATCTGACCGTCGATGTTATGCGTTCTGTCTATGTCCCGG GTAGTTCCTTCTTGCCAACCAAGAGGAACAATAGGATGAGGGAAGTCGACGGGGAAATTAA GGACAGATTGAGTGGCATTATTAACTCTAGGGTCAAGGCGATGAAAGCAGGCGAACCATCA GGAGAGGATTTGCTGGGCACCCTACTAGAATCCAATTTTAAAGAGATAGAGAGATTAGGGAA TAAAAAAAATGCTGGGATGTCAATTGAAGATGTAATTAGCGAATGCAAACTGTTCTACTTTGC CGGGCAGGAGACTACAGGCATACTTCTTACATGGACTTGCGTTTTACTTTCTCGTCACCCTG AATGGCAAGAGAGAGCACGTGAGGAGATCTTTCAAGTATTCGGAAACGGCAAAGTCGACTT CGATAGAGTACAGAATCTGAAAATTGTCCCGATGATCCTGTATGAAGTCCTGCGTTTGTACC CGCCAGTTATTGAACTAACTAAGGTCACTTACGAAGAGCAGAAGTTAGGCAACTTGACAATC CCCGCTGGGGTCCAACTAATGATGCCCTCCATTTTATTACACAGAGATCAAGAGATGTGGGG CGCGGATAGCAAAGAGTTTAATCCAGGGAGATTCGCCGATGGTATCTCAAAGGCGGTGAAA TCCCCGTTCTTCTATATCCCGTTCTCTTGGGGTCCTAGGATTTGCGTTGGGCAGAATTTTGC ACTGCTTCAAGCTAAGATGGCCTTAACGATGATCCTACAAAGATTCACCTTTGATTTATCTCC CACTTACGCGCATGCACCATTTACGGTCTTGACACTACAACCGCAACATGGAGCACAGGTC GTCTTTAGGAAAATCAAGTGCTAG
Ca610	ATGAAGATGGAAGTCATGCATATGTCAGTTGCCGCCTCACTAGCTGTGGTGTTCTTGGTCTG TATCTGGAGGGCGCTGAATTGGGCATGGTTCATGCCCAAAAAGATAGAGAAGAGACTGCGT CAGCAGGGCTTTAATGGTAATCCATACCGTCTGTTAGTTGGCGACTTAAAAGAATCCTCAAT GATGTTAAAAGAAGCTATGAGCAAGCCTATTCCCGTTTCCCAGGATATTGTGCAGCGTCTGA TGCCCCATGTAGTGAAAACCATCCAAACCTACGGAAAGAACTCTTTTACTTGGATTGGTAGA

	ATGCCCAGGGTCCATATCATGGAGCCAGAATTAATCAAAGATATCTTAGCCAACCACAATAAT TTCCAGAAGAATCATCACGCCTACAATCCGCTTACAAAGTTCTTGCTAACAGGCATCGGGAG CCTGGAGGGAGAGAAATGGGCAAAACATAGGAGAATCATCAGTCCGAGCTTTCATTTGGAA AAATTGAAAACAATGCTACCCGCGTTCTATGTTTCTTATGATGAGTTGTTAGGCAAGTGGGAA AGAGAGTCCTCCACAAAGGGGTCTGTAGAAGTAGACCTATTCCCGACATTCGATACGTTGAC ATCCGATGTCATTTCCAGGGTGGCGTTCGGATCATCATATGGGGAGGGCGGGCGTATCTTC ATCTTGTTGAAGGAACTGATGGATCTTACAGTAGACGTAATGCGTTCCGTCTATGTGCCAGG TTGGAGCCTGTTACCTACCAAGAGAAACCAGAGAATGAGGGAGGTCGATCGTGAGATACGT GAGAGATTGAGCGGAATAATTAATAGCAGAGTAAAAGCCATGAAGGCTGGTGAACCATCAG GTGACGACCTATTAGGGACTTTACTAGAGAGTAACTTTAGGGAAATCGAGCGTCTGGGGAAT AAGAAAAACGCTGGTATGAGTATCGAAGACGTCATCTCAGAGTGTAAACTTTTCTACTTTGCG GGCCAAGAGACTACCGGAATACTGCTAACATGGACTTGCGTAATATTATCAAGACATCCTGA ATGGCAGGAACGTGCGCGTGAAGAAATTTTTCAAGTATTTGGGAATGGGAAATTAGATTTTG ATCGTGTCCAAGGACTAAAAATAGTTCCTATGATTCTGTACGAGGTACTAAGACTTTACCCAC CTGTCATTGAGCTTACAAAGGTCACATATGAGGAACAAAAACTAGGAAATTTAACAATACCCG CTGGCGTGCAACTTATGATGCCCTCCATCCTTCTACATAGGGATAAAGAAATGTGGGGAGAT GACGCTACAGAATTCAATCCAGGTAGATTCGCAGAAGGAGTCGCCAAAGCGGTGAAGTCAC CGTTTTTTTATATCCCCTTCAGCTGGGGTCCCAGAATTTGTGTCGGCCAGAATTTCGCATTAC TACAAGCGAAAATGGCGTTAGCGATGATTCTGCAGAGATTTAGTTTCGATTTATCTCCGACTT ACGCTCATGCTCCGTTTACGGTGCTTACCTTGCAACCACAACACGGCGCTCAAGTAATTTTT AGACGTCTTAAGTGTTAG
Ti17-7DLH	ATGGAGGCAAACTTCAAACTAGTCGCGGTACTAGGGTTTACTTGTCTGGCACTGTATTGGGT TTATAGGGTCCTGGATTGGGTCTGGTTCAAGCCGAAAAAACTTGGAAAGTGTTTGAGGGAGC AGGGTTTTCGTGGAAACTCCTACAGACTTTTCCTAGGCGACCAGTATGAATCTGGAAAGTTG ATAAGAGAAGCCATGAGTAAACCAATCGGTGTAGAGGAAGACGTGAAAAAGAGGATAATTCC CCACATTCTTAGGACAGTCGAGACTCACGGGAAGAATTCCTTTATGTGGGTTGGACGTATTC CCAGAGTCCATATCACAGATCCAGAGCTAATAAAGGAGGTCCTTACTAAATATTATAAATTTC AAAAAAATCACCACGACCTAGACCCTATAACTAAGTTCTTGCTAACCGGCATAGGTTCTCTG GAAGGTGAACCATGGGCGAAAAGAAGGAAGATCATAAACGCAGCGTTTCATTTTGAAAAGTT AAAGCTGATGTTGCCCGCATTTTATCTGAGTTGTAGAGACATGGTTAGCAAGTGGGACAAAA AGGTGCCCGAAGGTGGTAGTCTTGAGGTTGATGTGTGGCACGACATTGAAACTCTGACCGG TGACGTCATTTCCAGAACTCTATTCGGTAGCAATTACGAGGAGGGGAGACGTATCTTTGAGC TAATGAAGGAGCTAACTGCGCTAACGATCGATGTTATTAGAAGCGTCTACATCCCGGGTCAA CGTTTTTTACCCACTAAAAGAAACAATAGAATGAGGGCGATAGACAAGGAGGTTAGAGTTAG AATCAAAGAAATTATAAACAACAAAACCAAAACTTTGAAAGCCGGAGTAGCGGCAAGCGACG ACTTCCTTGGAATATTACTAGAATGCAATCTTAACGAGATTAGAGAACAGGGCAACAACAAAA ATGCAGGTATGACAATAGAACAGATTATCGGGGAATGTAAATTGTTCTATTTCGCTGGTCAG GACACCACATCCACTTTACTAGTCTGGACAATGGTTCTATTATCCAGGTTTCCAGAGTGGCA GAACAGGGCGAGGGAGGAGGTGTTTCAGGTGTTCGGCAACAAGACGCCCGACTATGATGG CATTTCCCATTTAAAAATAGTGACCATGATCCTATATGAGGTGTTGAGACTATACACACCCGT AGCGGAGCTAACCAAGGTAGCGCACGAGGACACGCAGCTTGGAAAATATTTCATTCCAGCC GGTGTCCAATTAATGATGCCGCAAATGTTACTACACCATGACCCTCAGATCTGGGGCGAAGA TGTTATGGAGTTCAAACCAGAGAGATTTAGTGAGGGTGTGCTAAAAGCGACAAAGAGTCAGG GATCTTACTTTCCATTTAGCCTGGGACCAAGAATGTGTATAGGTCAAAATTTCGCACTTTTAG AAGCTAAAATGGCCATGGCATTGATCCTAAGGCGTTTTAGTTTCGAACTTTCCCCCTCTTACG TACATGCCCCATTTACATTAATCACGATGCAACCGCAATATGGGGCTCATCTAATCTTACACA AGCTTTAG
Ti18-7DLH	ATGGAAGCCAACTTTAAATTGGTGGCTGTGCTGGGATTCACGTCACTGGCGCTGTATTGGGT TTACAGGGTTCTGGACTGGGTTTGGTTCAAGCCGAAAAAGCTTGAAAAATGTCTAAGAGAAC AAGGGTTCAGGGGAAATAGTTACCGTCTTTTCCTGGGAGATCAGTACGAGTCTGGCAAGCT GATAAGGGAAGCGATGAGCAAACCAATAGGCGTCGAGGAAGACGTGAAGAAGAGGATAATC CCTCACATCTTGAGAACGGTCGAGACACACGGTAAGAACTCATTCATGTGGGTAGGTAGAAT ACCGAGGGTTCATATTACAGACCCGGAACTGATAAAAGAGGTATTGACCAAATACTATAAGT TTCAGAAGAATCACCATGACCTGGACCCCATTACAAAATTTCTATTGACGGGGATCGGCAGT TTGGAAGGGGAGCCCTGGGCCAAGAGGAGAAAGATTATAAATGCGGCGTTCCACTTCGAGA AGTTGAAGCTGATGTTGCCCGCTTTCTACTTAAGCTGTCGTGATATGGTAAGCAAGTGGGAT AAAAAAGTCCCTGAAGGCGGTTCCGCTGAAGTCGATGTTTGGCACGACATTGAAACCTTAAC AGGGGACGTGATCAGTAGGACCTTATTTGGGAGTAACTATGAAGAAGGTAGGAGAATATTTG AGCTTATGAAAGAGTTAACGTCCTTGACCATAGATGTTATCAGGTCAGTATATATACCGGGC CAACGTTTTCTACCTACGAAGCGTAATAACAGAATGCGTGCCATCGACAAGGAAGTCCGTGT TAGAATCAAAGAGATAATAAACAACAAGATGAAGACCCTTAAAGCGGGCGAGGCAGCAAGC

	GACGACTTCTTAGGTATTTTGCTAGAGTGCAATTTGAACGAAATTAGAGAACAGGGGAATAA CAAGAACGCCGGCATGACTATAGAACAAATTATAGGGGAGTGTAAACTTTTCTACTTTGCAG GACAAGACACCACCTCCACATTGCTAGTATGGACCATGGTGCTGCTGTCACGTTTTCCAGAG TGGCAAACGCGTGCTAGAGAGGAGGTCTTTCAAGTATTTGGCAACAAAACGCCAGACTACG ATGGCATATCCCATCTTAAGATAGTAACAATGATTTTATACGAGGTGCTTCGTCTATACACAC CTGTTGCAGAATTGACTAAAGTTGCACACGAGGATACACAACTTGGTAAATACTTTATTCCTG CTGGTGTTCAATTAATGATGCCGCAGATGCTTCTACATCACGACCCGCAAATCTGGGGCGAA GATGTGATGGAGTTCAAACCAGAGAGGTTTTCTGAAGGTGTTTTGAAAGCGACGAAGTCCCA AGGGAGTTACTTCCCATTCTCATTGGGGCCGCGTATGTGCATTGGTCAGAACTTTGCACTAT TGGAAGCGAAGATGGCTGTGGCACTAATCTTGCGTAGATTTTCATCAGAGCTTAGCCCCAGC TACGTACATGCACCATTTACTCTGATCACTATGCAACCAGAATACGGGGCACACCTTATCCTT AGGAAACTGTAG
Ug7DLH	ATGGGCGTCAACTTTAGTAGCGTCGCAATTCTTGGATTCATCTGCTTAGCGATCTACTGGTT CTACAGGGTTTTCGATTGGGCTTGGCTACGTCCTAAGAAGTTGGAGAAGTGTCTTCGTGAGC AAGGCTTCAAGGGTAATCCATATCGTCCGTTCCTTGGGGACCAGTATGAAAGCGGAAAACTA ATACGTGAAGCAATGAGCAAGCCGATTGGCGTTGAAGAGGACGTGAAGAAAAGGATCATTC CACACATATTGAAGACGGTTCAAACGCACGGAAAAAATTCTTTCATGTGGGTCGGAAGGATT CCAAGGGTTCACGTAACGGATCCGGAACTTATTAGGGAAGTCCTGACAAAATATTACAAGTT CCAGAAGAATCACCACGACCTGGATCCCATTACAAAATTCCTGTTGACAGGAATTGGGTCTT TGGAGGGGGATCCGTGGAGTCGTAGGAGAAAGATAATCAATTCAGCGTTCCAGTTCGAAAA ACTTAAGCTAATGTTGCCTGCATTCTACCTGTCATGTAGGGACATGGTGTCTAAGTGGGATA ATAAGGTCCCTGAGGGCGGCAGTGCTGAGTTAGATGTGTGGCATGATATAGAAACCCTTACT GGTGACGTCATAGCAAGAACATTGTTTGGGTCTAATTACGAAGAAGGAAAAAGAATATTCGA GCTTATTAAAGAATTAACCTCCCTGACAATCGATGTGATTAGATCTGTGTATATACCCGGACA GCGTTTCTTACCGACTAAGAGAAACAATAGAATGAGAGCAATAGATAAGGAAGTGAGGGTGA GAATCACTGAGATAATAAACAAAAAAATGAAGGCGATGAAAAACGGCGAAGCAACAGGGGAT AACTTCCTTGGGATCCTTCTAGAGTGCAACCTAAATGAAATAAAAGAGCATGGAAACAACAAA AACGCAGGCATGAGCATAGAGGACATCATCGGCGAGTGCAAACTGTTTTACTTCGCGGGAC AAGACACTACTAGTACGCTTATAGTTTGGACGATGGTGTTGCTATCCAGATTCCCCGAATGG CAACAACGTGCGAGGGACGAAGTTTTACAGGTATTCGGAGACCGTAAACCTGACTATGACG GTATAAGCAGACTGAAGATAGTGACAATGATTTTGTATGAGGTCTTAAGGATTTATTCTCCCG TCGCGGAATTAACGAAAGTCGCACATGAGGATACCCAGCTAGGCAAATACTTCATCCCTGCT GGGGTTCAACTAATGATGCCTCAGATGTTATTACATCATGACCCCGACATATGGGGAGACGA TGTGATGGAATTTAAGCCAGAGAGATTCTCAGAGGGCGTCCTAAAGGCGACGAAGTCCCAA GGGAGTTATTTCCCTTTCAGTCTTGGACCACGTATGTGTATCGGGCAGAATTTTGCCCTTCTA GAGGCTAAAATGGCCATGGCTTTGATCCTTAGGCGTTTCAGTTTTGAGCTATCTCCATCATAT GTACATGCACCTTTTACCCTTATCACCATGCAGCCCCAGTATGGAGCACACCTGACGCTACA CAAGTTAGAAAATCAGAAAATGTTGCTTTAG
ScGDP1	ATGCCGCCAGCTAGTACTAGTACTACCAATGATATGATAACCGAAGAACCTACTTCTCCACA CCAAATCCCAAGGCTTACAAGGAGACTTACGGGGTTTCTTCCCCAAGAAATCAAGTCAATTG ACACGATGATTCCTTTAAAGTCAAGAGCGTTATGGAATAAGCATCAAGTCAAAAAATTTAACA AGGCAGAAGATTTTCAAGATAGATTCATTGACCATGTGGAAACTACATTAGCACGTTCCCTAT ATAATTGTGATGACATGGCTGCTTATGAAGCTGCTTCGATGAGTATTCGTGACAATTTGGTCA TTGACTGGAACAAAACTCAGCAGAAATTCACCACAAGAGACCCAAAGAGAGTTTACTATTTGT CTTTGGAGTTTTTGATGGGTAGGGCTTTGGATAATGCCCTGATTAATATGAAGATTGAAGATC CGGAAGACCCTGCTGCCTCAAAGGGAAAACCAAGAGAAATGATTAAAGGGGCTTTGGATGA TTTAGGTTTCAAGTTAGAGGATGTCTTGGACCAAGAACCGGACGCAGGTTTAGGTAATGGTG GTCTAGGTCGTCTTGCAGCTTGCTTCGTCGACTCAATGGCAACGGAAGGCATCCCTGCCTG GGGTTATGGTCTACGTTATGAGTATGGTATCTTTGCTCAAAAGATTATTGACGGTTACCAGGT GGAAACTCCAGATTACTGGTTAAATTCTGGTAATCCATGGGAAATTGAACGTAACGAAGTGC AAATTCCTGTCACCTTTTATGGTTATGTTGATAGACCAGAAGGCGGTAAAACTACACTGAGTG CGTCACAATGGATCGGTGGGGAAAGAGTTCTTGCTGTCGCGTATGATTTCCCAGTTCCGGG TTTCAAGACTTCCAATGTAAATAACTTAAGACTATGGCAAGCAAGGCCAACAACAGAATTTGA TTTTGCAAAATTCAATAATGGTGACTATAAAAACTCTGTGGCTCAGCAACAACGCGCAGAGTC TATAACCGCTGTGTTGTATCCAAACGATAACTTTGCTCAAGGTAAGGAGTTGAGGTTGAAAC AGCAGTACTTCTGGTGTGCTGCATCCTTACACGACATCTTAAGAAGATTCAAAAAATCCAAGA GGCCATGGACTGAATTTCCTGACCAAGTGGCTATTCAGTTGAATGATACTCATCCAACTTTAG CCATCGTTGAATTACAGAGAGTTTTGGTCGATCTAGAAAAACTAGATTGGCACGAGGCTTGG GACATCGTGACCAAGACTTTTGCTTATACTAACCACACTGTTATGCAAGAGGCCCTGGAAAA ATGGCCCGTCGGCCTCTTTGGCCATTTGCTACCCAGACATTTGGAAATTATATATGATATCAA CTGGTTCTTCTTGCAAGATGTGGCCAAAAAATTCCCCAAGGATGTTGATCTTTTGTCTCGTAT

	ATCCATCATCGAAGAAAACTCTCCAGAAAGACAGATCAGAATGGCCTTTTTGGCTATTGTTG GTTCACACAAGGTTAATGGTGTTGCTGAATTGCACTCTGAATTAATCAAAACGACCATATTTA AAGATTTTGTCAAGTTCTATGGTCCATCAAAGTTTGTCAATGTCACTAACGGTATCACACCAA GGAGATGGTTGAAGCAAGCTAACCCTTCATTGGCTAAACTGATCAGTGAAACCCTTAACGAT CCAACAGAGGAGTATTTGTTGGACATGGCCAAACTGACCCAGTTGGGAAAATATGTTGAAGA TAAGGAGTTTTTGAAAAAATGGAACCAAGTCAAGCTTAATAATAAGATCAGATTAGTAGATTT AATCAAAAAGGAAAATGATGGAGTAGACATCATTAACAGAGAGTATTTGGACGACACCTTGTT TGATATGCAAGTTAAACGTATTCATGAATATAAGCGTCAACAGCTAAACGTCTTTGGTATTAT ATACCGTTACCTGGCAATGAAGAATATGCTGAAGAACGGTGCTTCGATCGAAGAAGTTGCCA AGAAATATCCACGCAAGGTTTCAATCTTTGGTGGTAAGAGTGCTCCTGGTTACTACATGGCT AAGCTGATCATAAAATTGATCAACTGTGTTGCTGACATTGTTAATAACGACGAGTCAATTGAG CATTTGTTGAAGGTTGTCTTTGTTGCTGATTATAATGTTTCTAAGGCTGAAATCATTATTCCAG CAAGTGACTTGAGTGAGCATATTTCTACTGCTGGTACTGAAGCGTCTGGTACTTCTAATATGA AGTTTGTTATGAACGGTGGTTTGATTATTGGTACTGTTGATGGTGCCAATGTGGAAATCACAA GGGAAATTGGTGAAGATAATGTCTTCTTGTTTGGTAACCTAAGTGAAAATGTCGAAGAATTGA GATACAACCATCAATACCATCCACAAGATTTACCATCTAGTTTGGATTCTGTTTTATCCTACAT TGAAAGTGGACAATTTTCTCCAGAAAATCCAAATGAATTCAAACCTTTAGTCGACAGTATTAA GTACCACGGCGATTATTACCTGGTCAGTGATGACTTTGAATCCTATCTGGCCACCCATGAAT TAGTGGACCAGGAGTTCCACAATCAAAGGTCAGAATGGTTAAAAAAGAGTGTCCTGAGCGTT GCAAACGTCGGCTTCTTTAGCAGTGATCGTTGTATCGAGGAATACTCCGATACCATTTGGAA CGTTGAACCAGTGACTTAG
ScUGP1	ATGTCCACTAAGAAGCACACCAAAACACATTCCACTTATGCATTCGAGAGCAACACAAACAG CGTTGCTGCCTCACAAATGAGAAACGCCTTAAACAAGTTGGCGGACTCTAGTAAACTTGACG ATGCTGCTCGCGCTAAGTTTGAGAACGAACTGGATTCGTTTTTCACGCTTTTCAGGAGATATT TGGTAGAGAAGTCTTCTAGAACCACCTTGGAATGGGACAAGATCAAGTCTCCCAACCCGGAT GAAGTGGTTAAGTATGAAATTATTTCTCAGCAGCCCGAGAATGTCTCAAACCTTTCCAAATTG GCTGTTTTGAAGTTGAACGGTGGGCTGGGTACCTCCATGGGCTGCGTTGGCCCTAAATCTG TTATTGAAGTGAGAGAGGGAAACACCTTTTTGGATTTGTCTGTTCGTCAAATTGAATACTTGA ACAGACAGTACGATAGCGACGTGCCATTGTTATTGATGAATTCTTTCAACACTGACAAGGATA CGGAACACTTGATTAAGAAGTATTCCGCTAACAGAATCAGAATCAGATCTTTCAATCAATCCA GGTTCCCAAGAGTCTACAAGGATTCTTTATTGCCTGTCCCCACCGAATACGATTCTCCACTG GATGCTTGGTATCCACCAGGTCACGGTGATTTGTTTGAATCTTTACACGTATCTGGTGAACT GGATGCCTTAATTGCCCAAGGAAGAGAAATATTATTTGTTTCTAACGGTGACAACTTGGGTG CTACCGTCGACTTAAAAATTTTAAACCACATGATCGAGACTGGTGCCGAATATATAATGGAAT TGACTGATAAGACCAGAGCCGATGTTAAAGGTGGTACTTTGATTTCTTACGATGGTCAAGTC CGTTTATTGGAAGTCGCCCAAGTTCCAAAAGAACACATTGACGAATTCAAAAATATCAGAAAG TTTACCAACTTCAACACGAATAACTTATGGATCAATCTGAAAGCAGTAAAGAGGTTGATCGAA TCGAGCAATTTGGAGATGGAAATCATTCCAAACCAAAAAACTATAACAAGAGACGGTCATGA AATTAATGTCTTACAATTAGAAACCGCTTGTGGTGCTGCTATCAGGCATTTTGATGGTGCTCA CGGTGTTGTCGTTCCAAGATCAAGATTCTTGCCTGTCAAGACCTGTTCCGATTTGTTGCTGG TTAAATCAGATCTATTCCGTCTGGAACACGGTTCTTTGAAGTTAGACCCATCCCGTTTTGGTC CAAACCCATTAATCAAGTTGGGCTCGCATTTCAAAAAGGTTTCTGGTTTTAACGCAAGAATCC CTCACATCCCAAAAATCGTCGAGCTAGATCATTTGACCATCACTGGTAACGTCTTTTTAGGTA AAGATGTCACTTTGAGGGGTACTGTCATCATCGTTTGCTCCGACGGTCATAAAATCGATATTC CAAACGGCTCCATATTGGAAAATGTTGTCGTTACTGGTAATTTGCAAATCTTGGAACATTGA
CrSGD	ATGGGGAGCAAAGACGACCAATCTTTAGTTGTCGCTATCAGCCCAGCGGCGGAACCGAATG GCAACCACAGTGTGCCCATACCCTTCGCCTACCCCTCCATTCCGATTCAGCCTAGAAAGCAT AACAAACCTATTGTACACAGGAGAGATTTTCCGAGTGATTTCATTCTTGGTGCAGGGGGTAG TGCATACCAATGCGAAGGCGCTTACAATGAGGGCAATAGAGGTCCTTCCATATGGGATACTT TTACCAACCGTTACCCTGCTAAGATTGCCGACGGAAGTAATGGTAACCAAGCAATTAACTCC TATAACTTATATAAGGAGGACATCAAAATCATGAAGCAAACAGGGTTGGAGTCTTATAGGTTT AGTATAAGTTGGTCCAGAGTGCTACCTGGTGGGAATCTTTCTGGGGGAGTAAATAAGGATG GGGTGAAGTTCTACCACGACTTTATCGATGAATTATTAGCTAACGGAATAAAGCCATTCGCTA CTCTGTTCCACTGGGATTTGCCACAGGCGCTGGAAGATGAATACGGCGGTTTCCTTTCAGAT CGTATTGTGGAAGACTTTACCGAATACGCGGAGTTCTGTTTCTGGGAATTTGGTGATAAGGT CAAATTCTGGACGACGTTCAACGAGCCCCATACATACGTAGCAAGTGGCTATGCGACGGGC GAGTTTGCGCCGGGCAGAGGAGGCGCAGATGGCAAGGGCGAACCGGGCAAAGAGCCGTA CATCGCGACTCACAACTTACTTCTATCCCACAAAGCTGCTGTTGAGGTGTACAGGAAAAACT TTCAAAAATGTCAAGGGGGGGAAATAGGTATTGTCCTGAATAGTATGTGGATGGAACCTCTA AACGAAACCAAAGAAGATATAGATGCGCGTGAAAGGGGTTTAGACTTCATGCTAGGCTGGTT TATCGAACCTCTTACAACGGGAGAATACCCCAAAAGTATGAGGGCGCTAGTCGGGTCCAGG

	TTACCCGAATTTAGCACCGAAGTTTCTGAAAAGCTTACTGGATGTTACGATTTTATTGGAATG AATTACTATACTACGACTTACGTATCTAATGCCGATAAGATCCCTGATACTCCGGGCTACGAA ACTGACGCTAGGATAAACAAAAATATTTTTGTGAAGAAAGTTGACGGGAAAGAGGTAAGAAT AGGGGAACCATGTTATGGCGGCTGGCAACACGTCGTACCGAGCGGATTATACAACTTACTA GTATACACTAAGGAAAAATACCACGTCCCAGTGATTTACGTGTCAGAGTGTGGTGTGGTTGA GGAAAATAGGACCAATATATTGTTAACAGAGGGAAAAACGAACATTCTGTTAACGGAAGCGA GACACGATAAGTTACGTGTGGACTTCTTACAGTCCCATCTTGCAAGCGTAAGAGATGCGATA GATGACGGCGTCAACGTGAAAGGTTTCTTCGTTTGGTCATTCTTCGATAACTTTGAATGGAAT CTAGGTTACATCTGTAGATATGGTATTATACACGTTGATTACAAGACCTTTCAGAGATACCCC AAAGACTCCGCCATCTGGTACAAGAATTTCATCTCCGAAGGCTTCGTAACAAACACGGCGAA AAAGAGATTTCGTGAAGAGGACAAGTTAGTGGAACTGGTGAAAAAACAGAAATATTAG
CaSGD	ATGGAAGCACAATCCATACCCTTATCAGTACACAATCCGAGCTCAATTCATAGACGTGACTTC CCACCGGATTTTATCTTCGGGGCTGCAAGTGCAGCTTATCAGTACGAAGGCGCAGCAAACG AGTACGGGCGTGGGCCGAGCATATGGGACTTCTGGACACAAAGACATCCAGGTAAGATGGT AGACTGCTCTAATGGTAACGTCGCCATCGATAGCTATCACAGATTTAAAGAAGATGTGAAGA TAATGAAGAAAATCGGCCTGGATGCATACAGGTTCAGTATTAGCTGGAGTAGGCTTTTGCCC TCTGGCAAACTTTCTGGCGGTGTTAATAAAGAGGGGGTGAATTTTTACAACGACTTCATCGA CGAACTGGTGGCAAACGGGATAGAGCCTTTTGTCACGTTATTTCATTGGGATCTACCACAGG CATTGGAGAACGAGTACGGGGGATTTCTTAGCCCAAGAATCATCGCCGATTATGTCGACTTC GCTGAGCTTTGCTTTTGGGAGTTTGGAGATAGAGTAAAAAATTGGGCAACATGCAATGAGCC GTGGACCTATACTGTGTCAGGATATGTTTTGGGTAATTTCCCTCCTGGGCGTGGCCCGTCTT CTCGTGAAACGATGCGTTCCCTGCCTGCTCTTTGTCGTCGTTCCATTTTACATACACATATAT GCACAGATGGGAATCCTGCTACCGAACCTTATAGAGTGGCCCACCATCTTCTATTGAGCCAC GCGGCGGCAGTTGAGAAGTACAGAACCAAGTATCAGACCTGTCAACGTGGGAAGATAGGAA TTGTTCTTAACGTTACGTGGCTTGAGCCCTTTTCAGAATGGTGTCCTAATGACCGTAAGGCA GCAGAGCGTGGGCTAGATTTTAAACTAGGGTGGTTCCTTGAGCCGGTTATCAATGGCGACT ATCCCCAATCCATGCAAAACTTAGTTAAGCAACGTCTGCCGAAGTTTAGCGAGGAGGAGAGC AAATTACTGAAAGGCAGTTTTGATTTTATTGGGATTAACTATTACACCTCAAACTATGCTAAAG ATGCCCCCCAAGCGGGGAGCGATGGAAAGCTATCTTACAACACAGATTCAAAAGTAGAGATT ACGCACGAACGTAAGAAGGACGTGCCTATTGGACCTCTTGGTGGCTCTAATTGGGTGTATCT TTACCCTGAAGGGATTTATAGGTTATTGGATTGGATGAGAAAAAAGTATAATAACCCATTGGT ATACATCACTGAGAATGGGGTAGATGATAAGAACGACACAAAATTAACTCTTAGCGAAGCAA GGCATGACGAGACGAGGCGTGACTACCACGAGAAGCATTTGCGTTTTTTGCATTATGCCAC GCATGAGGGCGCGAACGTGAAAGGCTATTTCGCTTGGAGTTTCATGGATAATTTCGAGTGG AGTGAAGGCTACAGCGTTAGATTTGGGATGATATACATCGATTATAAGAATGACTTGGCCCG TTATCCCAAAGACTCAGCCATTTGGTATAAGAACTTTCTAACTAAGACTGAGAAAACCAAGAA GCGTCAATTGGATCACAAAGAACTTGATAACATCCCGCAAAAGAAGTAG
GsSGD	ATGGCCACCCCGTCAAGCACAATAGTTCCAGACGCCACTAAAATAAATAGGCGTGATTTTCC CTCAGACTTTGTCTTTGGAGCAGCCAGTAGCGCTTATCAGATAGAAGGTGGGGCATCTGAG GGAGGACGTGGACCTTCAATTTGGGATACATTCACTAAAAGGAGGCCGGAAATGGTTAAAG GGGGGTCCAATGGGAATGTAGCGATTGATTCCTACCACTTGTATAAGGAGGACGTGAAGATT CTGAAGAATCTGGGGTTAGACGCGTATCGTTTTTCAATCTCTTGGAGCCGTATATTGCCCGG TGGTAACCTTTCCGGAGGTATTAACAAAGAGGGCATTGATTTCTATAATAATTTCATCGACGA ACTGATAGCTTCTGGTATCCAGCCGTATGTTACGTTATTTCACTGGGACGTCCCCCAAGCCT TAGAAGACGAGTATGGAGGTTTCCTGTCCCCCAAAATCGTTGATGACTTCAGAGATTATGCA GAGCTGTGCTTCTGGAATTTTGGGGATCGTGTCAAGAATTGGATCACATTAAATGAGCCCTG GACGTTCTCCGTCGATGGGTACGTTGCGGGTACATTCGCACCCGGAAGGGGGGCAACACC AACTGACCAGGTCAAAGGGCCTATAAAAAGACATAGGTGCTCAGGCTGGGGTCCTCAATGT TCCAACTCCGACGGGAATCCCGGGACAGAGCCTTACCTAGTCACCCATCACCAAATTCTAG CACATGCGGCCGCGGTTGAATCTTATAGAAATAAGTTCAAGGCGTCCCAAGAAGGCCAAATT GGCATTACTATCGTAGCGCAATGGATGGAGCCGTTAAACGAGAAAAGTGACAGCGACGTAC AGGCGGCAAAGCGTGCGCTAGACTTTATGTACGGATGGTTTATGGAGCCGATCACTTCTGG AGATTACCCCGAAATAATGAAAAAAATCGTCGGTAGCCGTCTTCCCAAGTTTTCTGCCGAAC AATCAAGAAAGCTAAAGGGAAGCTATGACTTTCTTGGCCTAAATTACTACACAGCAAATTATG TCACCAGCGCCCCCAACCCCACTGGTGGAATAGTAAGTTACGATACGGACACGCAGGTCAC TTATCACTCAGATAGGAATGGCAAGTTGATTGGGCCCTTGGCGGGGTCTGAATGGCTGCAC ATCTATCCCGAAGGTATCAGGAAGTTGCTGGTCTACACCAAAAAAACGTATAATGTTCCCTTG ATTTACATTACGGAAAACGGGGTCGACGAGCTGAACGACACAAGTTTGACGTTAAGTGAGG CCCGTGTTGACCCAATTCGTATCAAATTCATACAGGATCACCTACTACAACTTAGATTGGCCA TAGATGATGGCGTGAACGTGAAGGGCTACTTTGTCTGGTCCCTACTTGATAACTTTGAGTGG AATGAGGGTTTTACAGTGCGTTTTGGCATGATTCACGTTAATTATAATGATCAATATGCTAGA

	TATCCTAAGGATTCCGCAATCTGGTTGATGAATAATTTTCATAAAAAGTTTTCCGGGCCACCT GTGAAAAGAAGCGTAGAAGAAAACCAAGAGACGGACTCTCGTAAGCGTAGTCGTAAGTAG
RsSGD	ATGGACAACACGCAGGCGGAGCCACTGGTCGTGGCTATAGTCCCCAAGCCAAACGCCTCAA CGGAACATACGAATAGTCACTTAATTCCGGTCACGAGATCAAAAATTGTTGTTCACCGTAGA GATTTTCCCCAGGATTTTATCTTTGGCGCCGGAGGATCAGCCTACCAGTGCGAGGGGGCAT ACAACGAAGGGAACAGAGGTCCTTCCATCTGGGATACCTTTACGCAACGTTCACCAGCCAA GATCAGTGATGGTTCCAACGGCAATCAGGCTATAAACTGTTACCATATGTACAAGGAGGACA TTAAGATCATGAAACAGACAGGGCTTGAGAGTTATAGATTCAGTATTAGCTGGTCAAGGGTA TTGCCGGGCGGTAGGTTAGCAGCCGGCGTCAACAAAGACGGCGTTAAGTTTTACCACGATT TCATAGACGAATTATTAGCGAACGGGATAAAGCCTTCAGTCACTCTTTTTCACTGGGATCTGC CTCAAGCATTGGAAGACGAGTATGGAGGATTTTTATCACACAGGATTGTTGACGATTTTTGC GAGTATGCCGAATTCTGTTTTTGGGAGTTCGGAGACAAAATAAAATATTGGACAACCTTCAAT GAGCCACACACGTTCGCAGTAAACGGTTACGCACTGGGAGAGTTCGCGCCGGGTAGAGGA GGGAAGGGAGACGAGGGGGACCCAGCCATCGAGCCCTACGTTGTTACTCACAATATTCTGC TGGCGCACAAAGCTGCCGTTGAGGAGTACAGGAATAAATTTCAAAAGTGCCAGGAAGGGGA GATAGGGATTGTTCTTAATTCTATGTGGATGGAACCACTATCCGATGTTCAAGCCGATATCGA CGCACAAAAAAGGGCTTTGGACTTTATGTTGGGATGGTTCCTAGAGCCTTTAACAACAGGGG ACTACCCGAAATCAATGCGTGAACTAGTCAAAGGGAGACTGCCGAAGTTTAGTGCAGATGAC TCAGAAAAGTTGAAGGGATGCTATGACTTCATTGGGATGAATTACTATACTGCTACTTATGTC ACGAATGCGGTCAAAAGCAACAGCGAAAAGCTGTCTTATGAAACCGATGATCAAGTCACGAA AACCTTTGAAAGAAACCAGAAACCCATTGGCCATGCTTTATACGGTGGGTGGCAGCACGTC GTCCCCTGGGGACTTTACAAGCTGTTGGTGTACACGAAAGAAACTTACCACGTTCCAGTTTT ATATGTTACTGAGTCTGGTATGGTCGAAGAAAATAAGACCAAGATCTTGCTTTCCGAAGCAA GAAGGGATGCCGAACGTACGGATTACCATCAGAAGCATCTGGCTTCCGTCCGTGACGCCAT AGACGACGGGGTAAACGTAAAGGGATACTTCGTATGGAGTTTTTTCGATAATTTTGAATGGA ACCTGGGGTATATATGTCGTTACGGAATCATTCACGTAGACTACAAGTCATTTGAGAGATATC CGAAAGAATCTGCTATCTGGTATAAGAACTTTATAGCCGGTAAGAGCACCACTTCACCCGCT AAACGTCGTCGTGAAGAGGCACAGGTGGAACTAGTTAAGAGGCAGAAGACTTAG
MsSGD	ATGGAAGCTAAAAGGTCTACAGCCGTCGTGTCTAATGACGCTTCCAAGATTAATCGTGGGGA CTTCGCTGAGGATTTTATATTTGGTGCTGCTTCATCTGCCTACCAGACGGAAGGGGGCGCTT CCGAAGGGGGACGTGGGCCAAGCATCTGGGATACCTTTACCCAGAGAAGACCGGGTATGA TAAAGGAAGGCGGGAATGGTAATGTGGCCGTTGATTCTTACCACCAGTATAAGGAGGATATA AAAATTTTGAAGAACATGGGGTTGGATGCGTATAGGTTTAGTATCTCTTGGTCCCGTGTGCTT CCAGGTGGTAACCTTAATGCTGGAGTGAACAAAGAGGGGATTAATTATTACAATAATTTAATT GATGAGCTTCTTGCGAATGGCATAGAGCCATACGTAACGTTGTTTCATTGGGACGTACCGCA GGCCTTGGAAGACAAATATGGCGGGTTCTTGAGTTCCCAAATAGTTGATGATTTCAGAGAAT ACGTGGAGCTATGTTTCTGGGAATTCGGCGATAGGGTCAAACACTGGATAACGCTAAACGAA CCTTGGAGCTTTTCTGTCGGCGGGTACGTAAACGGCACATTCGCCCCCGGCAGGGGAGCTT CATCTAGCGAGCAAGAAAACGACCATCCCGCACCTGCTTTATTAAGCAGGTGTTCACCTTGG CAATCTCAATCCATCTCTTCCAATGGTAACCCTGGTACCGAGCCTTATGTCGTTACGCACAA CCAGTTATTAGCCCACGCTGCCGCGGTGGAATTATATAAATCTAACTTCCAAAAATCTCAATC AGGTAAGATCGGAATAACACTTGTATCACAATGGATGGAGCCCCTGGACGAGAATAGCAAG GCCGATGTCGAAGCTGCTAAGAGGGCATTGGACTTCATGCTAGGATGGTTTATGGAACCCC TGACTACCGGAAACTATCCCAAGAGCATGAGAAAATTGGTAGGGAGCAGACTGCCAAAATTC TCTGCAGATCAGAGTAAGCAATTAAAAGGATCCTATGATTTCCTAGGCTTGAATTACTACACT GCGGATTACGTAACCTCCGCCTCCTCCTCAACTACGGGGGGGAACCTAAGTTATACAACAG ATTCCCAGGTGACGCACACCACAGATCGTAACGGAGTACCGATAGGTCCTCAGGGGGGTTC CGAGTGGTTGCATATTTACCCAGAGGGTATACGTAAATTGCTTGTTTACGTGAAGAAGACGT ATAATGTCCCCCTGATTTACATAACCGAAAACGGAGTCGACGAAGTAAATGACACATCTCTAA CCTTATCCGAGGCGAGGGTTGATAACACCAGAATTAAATATATCCAAGACCATTTGCTTAACA TTCGTCTAGCCATTTCTGATGGAGTTAATGTCAAGGGATACTTCGTGTGGTCTCTTCTGGATA ACTTCGAGTGGTCTGAGGGGTATACGGTTAGGTTTGGCTTCATTCACATAGACTATACGAAC AACTATGCCAGATACCCCAAGGACAGCGCAATATGGTTCATGAATAGTTTTCATAAAGAATAT CCAAAACAGTTTCTGAAAAGGACGTTGGAAGATCATGAAGATTTTGTTTCTAAAAAACGTCTT CGTCAGTAG
CrTHAS	ATGGCAATGGCGTCCAAATCACCTTCAGAGGAAGTATATCCTGTTAAGGCGTTTGGCCTTGC GGCGAAGGATTCTTCTGGGCTTTTCAGTCCGTTCAACTTTTCTCGTCGTGCGACAGGGGAG CATGACGTGCAGCTTAAAGTGCTTTACTGCGGGACCTGCCAGTATGACAGGGAGATGTCCA AGAATAAATTCGGATTCACTTCATATCCGTATGTGCTTGGGCACGAAATCGTCGGCGAGGTT ACGGAAGTTGGTTCAAAGGTCCAGAAATTTAAAGTTGGAGATAAGGTTGGAGTCGCCAGCAT AATAGAAACCTGTGGGAAGTGTGAAATGTGTACTAATGAAGTAGAAAATTATTGCCCAGAGG

	CCGGTTCCATCGACTCAAACTACGGTGCATGTAGTAACATAGCAGTGATAAATGAGAACTTT GTAATTAGGTGGCCCGAGAACCTACCGCTGGACAGTGGTGTGCCCCTTCTTTGTGCCGGAA TTACGGCCTACTCACCTATGAAAAGGTACGGACTAGATAAGCCGGGGAAACGTATTGGAATC GCAGGTCTAGGCGGTCTTGGCCACGTCGCGCTTCGTTTCGCGAAGGCCTTTGGCGCTAAG GTCACTGTTATATCTAGTTCCTTGAAGAAGAAGAGAGAAGCATTCGAGAAATTTGGAGCCGA TTCCTTTCTTGTGTCCTCCAACCCAGAAGAGATGCAGGGAGCCGCAGGTACTCTAGATGGTA TAATTGACACTATACCTGGTAATCACTCCCTGGAACCTCTACTTGCGTTACTTAAGCCACTGG GTAAACTGATAATTTTGGGCGCACCCGAAATGCCGTTTGAAGTCCCTGCCCCTTCTTTACTTA TGGGGGGCAAGGTCATGGCGGCCAGTACAGCGGGATCAATGAAAGAGATACAAGAAATGAT AGAATTTGCTGCCGAACATAATATAGTGGCAGACGTGGAAGTGATTTCAATCGACTATGTGA ACACTGCCATGGAGAGACTGGATAACAGCGATGTACGTTATCGTTTTGTAATTGATATAGGA AATACGCTGAAATCAAATTAG
CrSS	ATGGAGATTAGGGACCTGTTCTGGTCTCTACCAGCTATTGCTCTTTTGCAGGTCTTCCTATTC TTTCTGTTTAAAAACCCCAAAAAAACCACTTTAAAGTTACCGCCTGGTCCTCCCACCTTGCCC ATAATCGGTAATCTGCACCAGATGGCCTCCCCTCTACCACACAAGAAGTTGAAAGATTTAGC TGATAAATACGGACCGTTGATGCATCTTAAGTTGGGAGAAATAAGCACTGTTGTGATCAGTT CAAGCAGGTTGACAAAAGAATTCATGCAAACCCATGGGCTTAATTTCGCAGATCGTCCTCAG ACGGTTATAGCTAAGATCATGATGTATAATTGTTCCGGAGTGACACTTAGTATGTACGGTGAT TACTGGAGGAAACTTAGGCAAATATACGTGACGGAGTTATTAAACACTAAGTCAGTCCAGTC TTTCTCTTCAATAATGGAGGAGGAGCTTATTTTAATGGTTAAAAGTATTGAATCAGAAGTGGG AAAGCCAATGGAGTTAATAGAAAAGATCAGGTCCTATCTATTCGATACTTTATGTCGTTCAGC ACTGGGGAAGATTCATGGTAAAGGGAAGGAAACGCTGATCGAAATTTCTCGTGAAATGGTC GCACTGTCCGGGGTTCAGACTCTAGAAGACATTTTCCCCAGCGTAAAACTATTCAGCTATTT AAACCCACTAAGGCCTAAGGCCAAAAAGCTTTTTAAACGTCTGGATTCAGTGTTGGAGGACA TAATCAACCAGCAAGAAAACAAGCTTCTTTCTTTGAAGGATGGGGACAACCAACAGGAGAAA GAAGAAGATAACATGCTTAGCGTATTGCTTAGGTTAAGGAACGGGAAAGACTCAAAAGTGAA ACTAACAAACAATGACATCAAAGCTATTATATTCGAGCTATTTGTAGGCGGTATTAGTACGAG CTCCACGACAATCGAATGGGCGATGTCCGAGCTAATGAAAAACCCGGAAATGATGGAGAAG GGAAAGCATGAGGTACGTCAAGTTCTTAAAGGAAAGAAGAGAGTTTGTCAAATTGATGTCGA AAACATGTCCTACATCAAACTTGTATTAAAAGAAACGTTGAGGTTCCACCCCCCAGGACCAC TTCTGTTCCCCAGAAAATCTAGGGAACAATGCGAGATAGATGGCTATACGATCCCGGCGAAG GCCATGATTCTGATCAACAACTGGGTGCTTGGTCGTGATCCCGAGTACTGGGTGGACCCAG AAAAATTTGAGCCGGAACGTTTCAAGGACAACTTGGTAGACTATAAGGGCAACCATTTTGAA TTAATACCTTTTGGGGTCGGGCGTAGAATCTGCCCTGGCATATCCTTTGCAGTTACCAATATT GAGTTGTTGCTGGCTGCCCTATTATTTCATTTCGATTGGAAGCTACCCCATGGTATGGATCC GAAGGACCTAGACATGATTGAGCTATATAGGAGTGGTTGTACCAGGAAAAACCCCCTTGTTC TTATCCCGAAAATCTACATCCCGACAGGTGATGAGAATTAT
MsDCS1	ATGGCCGGTAAGTGTGCCCAGGAGGAGCACACAGTTAAGGCATTTGGTTGGGCAGCCAGG GAGGCGAGCGGTGCTCTGAGTCCTTATGGGTTTTCCAGGAGGGCGACCGGTGAGAGAGAT GTTAGGGTGAAAATACTATATTGCGGTATTTGCCGTACAGATGCTGAGATGATCAGTGATAA GTTTTGTTTCACCAAGTACCCACATGTGCCGGGGCATGAAATAGTTGGGGTGGTAAGTGAG GTAGGAAATAAAGTTCAAAAATTTAAAGTGGGTGCTAAGGTAGGGGTTACTGGCATAATCGG TTGTTGTAGAACTTGTTACAGTTGTACGAACGGATTGGAGTCTTACTGTCCGAATGTGGCCC TTACAGAAGCGGGCGAGGGGGGCTGTAGCAATTTTATCGTCTTAGATGAGGACTTCGTTTTC AGGTGGCCGGAGAAACTGCCTCTGGACTTGGGTGCCCCACTATTGTGTGCAGGAGCCGCTT CTTACTCTCCCTTAAAAAATTTCGGGCTTGACAAGCCCGGTTTACATATTGGGATAGCGGGA CTTGGAGGCATGGGACACGTTGCTGTGAAATTCGCTAAAGCATTTGGGGCTAAGGTGACAG TCATCTCTACATCCGATAATAAAAAGGAAGAAGCCATCAAGAAATACGGAGCCGACGCATTT CTTAATTCTTCAAACCCCGAGCAGATGAGAGCAGCGGCAGGTACGCTAGCAGCCATTGTAG ACACCATCCCAAGCCCACATAGCCTGGTACCTCTTCTAGATCTTCTTCTGCCACACGGCAAA GTCATAGTGTTGGGGGCACCCTCCGAACCATTTGTGCTTCCTGTTATGCCATTATTACAAGG CGGGCGTGTGGTGGCAGGATCATCTGGCGCAAGTCTAAAACAGATTCAGGAGATGCTGGAC TTTGCGGCAGAACACAACATAGTAGCAGACGCCGAGGTCATCCCTATAGATTATATAAATAC AGCCATAAAGAGAATTGAAAAGGGGGATATTAAATACCGTTTTGTTGTCGATATCGGAAACA CCCTTAAGAGTGCT
MsEnolMT4	ATGCAACCACAGCGTGGAAGAAAAAGGGAGAGGGAACGTGACAGAGAAGAGATGGAATCC GTCCAATCTAACAGTTCATCTAGCGACCAGTTCGCAATGAAGGGCGGGGATGATGACTTCTC CTATACAAAAAACAGCACATGGCAGAGGGATGCAATTCAAGCCACAAAATTTTTCATACAAGA AAGTATAGCCGAAAAACTTGATGTAAACAAGTTTTGCGGTAAAGCGTTCTGTGTGGCGGATT TGGGTTGCAGTGTTGGACCTAATACCCTTATTGCAATGCAAAACATCGTGGAAGCGGTAGAG TTAAAGTTCAAGAACAGAAAGGGATTTCATTCTCCCACGATTCCCGAATTCCAAGTATTTTTC

	AACGATCATACTGTTAACGACTTTAACACCTTGTTTCGTAGCCTTCCGACGGGACATGACAAA AGGTATTATGGCGTCGGTGTGCCCGGGTCTTTTTATGGGAGGTTATTTCCGTGTGATAGCAT ACATATAATGCACACATCTTTCTCAACTCCGTTTCTAAGTCAAGTGCCAAAGGAGGTAATCGA TAAAAACTCTGCGGCCTGGAATAAGGGTCGTATTCACCACAATTACGCAAAAGCTGACGTGC TAAAGGCTTACGAGGCACAACATGCTGAAGACATAGATTGTTTTCTGACGGCCAGGGCCAA GGAGCTGGTGCATGGAGGGTTGTTGATGGACGTAACCAGTTTCAGACCCGATGGGGTCCCT CACACACACGTCCTAACCAATATCGGGATGGAAGTACTAGGATACTGCTTGATGGATTTAGC GGGACTAATCGACGAGGAGAATGTTGATTCCTACAATGTTCCCGTTTATCTTCAGAGCCCCG AAGAATTAAAACAAGCAGTCCAGAGAAATAAATATTTCTCCATAGAAAAAATGGAGTCTGTTC CCATGATGATAGATTCAGACGTAAGTGCCAAAGCCCAGCAGTATTCTCTTGGCATGAGAGCC GTTATGGGAGATGTCATAAGAGAACAATTTGGTGCGGAAATCGTGGATAAACTGTTCGACTT GTTTAAGAAGAAGTTGGAAGAACACCCTAACTTTGCTAAGGGCGTTGTATTAGACATGTTTGT ACTATTAAAACGTAATGCGGAAGAC

Appendix C – Promoters and Terminators Used in this Study

Name	Sequence
ADH2 promoter	CAAAACGTAGGGGCAAACAAACGGAAAAATCGTTTCTCAAATTTTCTGATGCCAAGAACT CTAACCAGTCTTATCTAAAAATTGCCTTATGATCCGTCTCTCCGGTTACAGCCTGTGTAAC TGATTAATCCTGCCTTTCTAATCACCATTCTAATGTTTTAATTAAGGGATTTTGTCTTCATTA ACGGCTTTCGCTCATAAAAATGTTATGACGTTTTGCCCGCAGGCGGGAAACCATCCACTT TACGAGACTGATCTCCTCTGCCGGAACACCGGGCATCTCCAACTTATAAGTTGGAGAAAT AAGAGAATTTCAGATTGAGAGAATGAAAAAAAAAAAAAAAAAAAAGGCAGAGGAGAGCAT AGAAATGGGGTTCACTTTTTGGTAAAGCTATAGCATGCCTATCACATATAAATAGAGTGCC AGTAGCGACTTTTTTCACACTCGAAATACTCTTACTACTGCTCTCTTGTTGTTTTTATCACT TCTTGTTTCTTCTTGGTAAATAGAATATCAAGCTACAAAAAGCATACAATCAACTATCAACT ATTAACTATATCGTAAT
ICL1 promoter	ATTTATTGAAAAGTAAATATCTCGTAACCCGGATGCTTTGGGCGGTCGGGTTTTGCTACTC GTCATCCGATGAGAAAAACTGTTCCCTTTTGCCCCAGGTTTCCATTCATCCGAGCGATCA CTTATCTGACTTCGTCACTTTTTCATTTCATCCGAAACAATCAAAACTGAAGCCAATCACC ACAAAATTAACACTCAACGTCATCTTTCACTACCCTTTACAGAAGAAAATATCCATAGTCC GGACTAGCATCCCAGTATGTGACTCAATATTGGTGCAAAAGAGAAAAGCATAAGTCAGTC CAAAGTCCGCCCTTAACCAGGCACATCGGAATTCACAAAACGTTTCTTTATTATATAAAGG AGCTGCTTCACTGGCAAAATTCTTATTATTTGTCTTGGCTTGCTAATTTCATCTTATCCTTT TTTTCTTTTCACACCCAAATACCTAACAATTGAGAGAAAACTCTTAGCATAACATAACAAAA AGTCAACGAAAA
MLS1 promoter	GGCCGATGAAGTTAGTCGACGGATAGAAGCGGTTGTCCCCTTTCCCGGCGAGCCGGCA GTCGGGCCGAGGTTCGGATAAATTTTGTATTGTGTTTTGATTCTGTCATGAGTATTACTTA TGTTCTCTTTAGGTAACCCCAGGTTAATCAATCACAGTTTCATACCGGCTAGTATTCAAAT TATGACTTTTCTTCTGCAGTGTCAGCCTTACGACGATTATCTATGAGCTTTGAATATAGTTT GCCGTGATTCGTATCTTTAATTGGATAATAAAATGCGAAGGATCGATGACCCTTATTATTA TTTTTCTACACTGGCTACCGATTTAACTCATCTTCTTGAAAGTATATAAGTAACAGTAAAAT ATACCGTACTTCTGCTAATGTTATTTGTCCCTTATTTTTCTTTTCTTGTCTTATGCTATAGTA CCTAAGAATAACGACTATTGTTTTGAACTAAACAAAGTAGTAAAAGCACATAAAAGAATTA AGAAA
PCK1 promoter	CAATAGGAAAAAACCGAGCTTCCTTTCATCCGGCGCGGCTGTGTTCTACATATCACTGAA GCTCCGGGTATTTTAAGTTATACAAGGGAAAGATGCCGGCTAGACTAGCAAGTTTTAGGC TGCTTAACATTATGGATAGGCGGATAAAGGGCCCAAACAGGATTGTAAAGCTTAGACGCT TCTGGTTGGACAATGGTACGTTTGTGTATTAAGTAAGGCTTGGCTGGGGATAGCAACATT GGGCAGAGTATAGAAGACCACAAAAAAAAGGTATATAAGGGCAGAGAAGTCTTTGTAATG TGTGTAACTTCTCTTCCATGTGTAATCAGTATTTCTACTTACTTCTTAAATATACAGAAGTA AGACAGATAACCAACAGCCTTTCCCAGATATACATATATATCTTTATTTCAGCTTAAACAAT AATTATATTTGTTTAACTCAAAAATAAAAAAAAAAAACCAAACTCACGCAACTAATTATTCC ATAATAAAATAACAAC
Bay_ADH2 promoter	GATCCAGTTCTCCAGTGACACAGCCTTTATCTGGTCAAACCTTTCTTTCTAATCACCTATG CTGATGCTTAATTAAGGGATTTTTGTCTCCATCAACGGCATGCGCCCAAAAATGACGTTTT

	TTTTAACCCATAGACACGAAACTACCCATTTTCCACCGGCCTGACCTACCACCGGAACAA CGGCCATCTCCAACTTGCAAGTTGGGGAAATTAAGAGCATCGCAGGTTTAATGGAAGAAA AAAAAAAGGTACAGCACAGCGCAAATGGAGTTAGTTCCCTTATGTCACACACTCACACAC AGTCGGTCAGATCAAGCATACTGGGTGCGTATAAATAGAGTGGCCATTGCCACCCTGTTT ATCTCAAAATCTGTCTTGTTAGTGGTCTTCTCCCTTTTTCAGGTTACAATTCTCTTGTTTCT ACTTAGTATATAAGTATATCAAGCTATATTAAGCATACTATCAACTGTCAACTCTATCCTCA AAATACAATACAAA
TEF1 promoter	CCGCGAATCCTTACATCACACCCAATCCCCCACAAGTGATCCCCCACACACCATAGCTTC AAAATGTTTCTACTCCTTTTTTACTCTTCCAGATTTTCTCGGACTCCGCGCATCGCCGTAC CACTTCAAAACACCCAAGCACAGCATACTAAATTTCCCCTCTTTCTTCCTCTAGGGTGTCG TTAATTACCCGTACTAAAGGTTTGGAAAAGAAAAAAGAGACCGCCTCGTTTCTTTTTCTTC GTCGAAAAAGGCAATAAAAATTTTTATCACGTTTCTTTTTCTTGAAAATTTTTTTTTTTGATT TTTTTCTCTTTCGATGACCTCCCATTGATATTTAAGTTAATAAACGGTCTTCAATTTCTCAA GTTTCAGTTTCATTTTTCTTGTTCTATTACAACTTTTTTTACTTCTTGCTCATTAGAAAGAAA GCATAGCAATCTAATCTAAGTTTTAATTACAAA
PGK1 promoter	AGGCATTTGCAAGAATTACTCGTGAGTAAGGAAAGAGTGAGGAACTATCGCATACCTGCA TTTAAAGATGCCGATTTGGGCGCGAATCCTTTATTTTGGCTTCACCCTCATACTATTATCA GGGCCAGAAAAAGGAAGTGTTTCCCTCCTTCTTGAATTGATGTTACCCTCATAAAGCACG TGGCCTCTTATCGAGAAAGAAATTACCGTCGCTCGTGATTTGTTTGCAAAAAGAACAAAAC TGAAAAAACCCAGACACGCTCGACTTCCTGTCTTCCTATTGATTGCAGCTTCCAATTTCGT CACACAACAAGGTCCTAGCGACGGCTCACAGGTTTTGTAACAAGCAATCGAAGGTTCTG GAATGGCGGGAAAGGGTTTAGTACCACATGCTATGATGCCCACTGTGATCTCCAGAGCA AAGTTCGTTCGATCGTACTGTTACTCTCTCTCTTTCAAACAGAATTGTCCGAATCGTGTGA CAACAACAGCCTGTTCTCACACACTCTTTTCTTCTAACCAAGGGGGTGGTTTAGTTTAGTA GAACCTCGTGAAACTTACATTTACATATATATAAACTTGCATAAATTGGTCAATGCAAGAAA TACATATTTGGTCTTTTCTAATTCGTAGTTTTTCAAGTTCTTAGATGCTTTCTTTTTCTCTTT TTTACAGATCATCAAGGAAGTAATTATCTACTTTTTACAACAAATAT
TDH3 promoter	ACAGTTTATTCCTGGCATCCACTAAATATAATGGAGCCCGCTTTTTAAGCTGGCATCCAGA AAAAAAAAGAATCCCAGCACCAAAATATTGTTTTCTTCACCAACCATCAGTTCATAGGTCC ATTCTCTTAGCGCAACTACAGAGAACAGGGGCACAAACAGGCAAAAAACGGGCACAACC TCAATGGAGTGATGCAACCTGCCTGGAGTAAATGATGACACAAGGCAATTGACCCACGC ATGTATCTATCTCATTTTCTTACACCTTCTATTACCTTCTGCTCTCTCTGATTTGGAAAAAG CTGAAAAAAAAAGGTTGAAACCAGTTCCCTGAAATTATTCCCCTACTTGACTAATAAGTATA TAAAGACGGTAGGTATTGATTGTAATTCTGTAAATCTATTTCTTAAACTTCTTAAATTCTAC TTTTATAGTTAGTCTTTTTTTTAGTTTTAAAACACCAAGAACTTAGTTTCGAATAAACACACA TAAACAAACAAA
SPG5 terminator	CAAAGACGTTGTTTCATCGCGCTATTACCAAGAAGGTTACTTTACTTGTTCTTGCACATGG ACGCACGTTGTGTGTTCATATATATATATATATATATATATATTTGTGCTTGTTTTCATTGTC TCTATAGTTAATACATTCTATTTTTATCGTTATATTTGCATTCTCTTCGCATAAAAACTTCAT GAAAATTCGGCAGAAAATAAGC
IDP1 terminator	TCGAATTTACGTAGCCCAATCTACCACTTTTTTTTTTCATTTTTTAAAGTGTTATACTTAGTT ATGCTCTAGGATAATGAACTACTTTTTTTTTTTTTTTTTTTACTGTTATCATAAATATATATAC CTTATTGTTGTTTGCAACCGTCGGTTAATTCCTTATCAAGGTTCCCCAAGTTCGGATCATT ACCATC
PRM9 terminator	GACAGAAGACGGGAGACACTAGCACACAACTTTACCAGGCAAGGTATTTGACGCTAGCA TGTGTCCAATTCAGTGTCATTTATGATTTTTTGTAGTAGGATATAAATATATACAGCGCTCC AAATAGTGCGGTTGCCCCAAAAACACCACGGAACCTCATCTGTTCTCGTACTTTGTTGTG ACAAAGTAGCTCACTGCCTTATTATCACATTTTCATTATGCAACGCTTCGGAAAATACGAT GTTGAAAATGCC
CPS1 terminator	GCGCAATGATTGAATAGTCAAAGATTTTTTTTTTTTAATTTTTTTTTTTTTAATTTTTTTTTTTT TTCATAGAACTTTTTATTTAAATAAATCACGTCTATATATGTATCAGTATAACGTAAAAAAAA AAACACCGTCAGTTAAACAAAACATAAATAAAAAAAAAAAGAAGTGTCAAATCAAGTGTCA AAT
CYC1 terminator	TCATGTAATTAGTTATGTCACGCTTACATTCACGCCCTCCCCCCACATCCGCTCTAACCGA AAAGGAAGGAGTTAGACAACCTGAAGTCTAGGTCCCTATTTATTTTTTTATAGTTATGTTA GTATTAAGAACGTTATTTATATTTCAAATTTTTCTTTTTTTTCTGTACAGACGCGTGTACGC ATGTAACATTATACTGAAAACCTTGCTTGAGAAGGTTTTGGGACGCTCGAAGGCTTTAATT TGC

8. REFERENCES

1. Kavšček, M., Stražar, M., Curk, T., Natter, K. & Petrovič, U. Yeast as a cell factory: Current state and perspectives. *Microbial Cell Factories* vol. 14 94 (2015).

2. Day, K. J., Casler, J. C. & Glick, B. S. Budding Yeast Has a Minimal Endomembrane System. *Dev. Cell* **44**, 56-72.e4 (2018).

3. Zhang, X. & Li, S. Expansion of chemical space for natural products by uncommon P450 reactions. *Nat. Prod. Rep.* **34**, 1061–1089 (2017).

4. Srinivasan, P. & Smolke, C. D. Biosynthesis of medicinal tropane alkaloids in yeast. *Nat. 2020 5857826* **585**, 614–619 (2020).

5. Galanie, S. & Smolke, C. D. Optimization of yeast-based production of medicinal protoberberine alkaloids. *Microb. Cell Fact.* **14**, 144 (2015).

6. Li, Y. *et al.* Complete biosynthesis of noscapine and halogenated alkaloids in yeast. *Proc. Natl. Acad. Sci. U. S. A.* **115**, E3922--E3931 (2018).

7. Paddon, C. J. *et al.* High-level semi-synthetic production of the potent antimalarial artemisinin. *Nature* **496**, 528–532 (2013).

8. Brown, S., Clastre, M., Courdavault, V. & O'Connor, S. E. De novo production of the plant-derived alkaloid strictosidine in yeast. *Proc. Natl. Acad. Sci.* **112**, 3205–3210 (2015).

9. Zhang, J. *et al.* A microbial supply chain for production of the anti-cancer drug vinblastine. *Nature* **609**, 341–347 (2022).

10. Misa, J., Billingsley, J. M., Niwa, K., Yu, R. K. & Tang, Y. Engineered Production of Strictosidine and Analogues in Yeast. *ACS Synth. Biol.* **11**, 1639–1649 (2022).

11. O'Connor, S. E. & Maresh, J. J. Chemistry and biology of monoterpene indole alkaloid biosynthesis. *Nat. Prod. Rep.* **23**, 532 (2006).

12. Rakumitsu, K., Sakamoto, J. & Ishikawa, H. Total Syntheses of (−)-Secologanin, (−)-5-Carboxystrictosidine, and (−)-Rubenine. *Chem. – A Eur. J.* **25**, 8996–9000 (2019).

13. Sakamoto, J., Umeda, Y., Rakumitsu, K., Sumimoto, M. & Ishikawa, H. Total Syntheses of (−)-Strictosidine and Related Indole Alkaloid Glycosides. *Angew. Chemie Int. Ed.* **59**, 13414–13422 (2020).

14. Anthony, S. M. *et al.* Total Synthesis of (−)-Strictosidine and Interception of Aryne Natural Product Derivatives "Strictosidyne" and "Strictosamidyne". *J. Am. Chem. Soc.* **143**, 7471–7479 (2021).

15. Geerlings, A. *et al.* Biotransformation of tryptamine and secologanin into plant terpenoid indole alkaloids by transgenic yeast. *Appl. Microbiol. Biotechnol. 2001 563* **56**, 420–424 (2001).

16. Kang, H. N. *et al.* In vitro biosynthesis of strictosidine usinglonicera japonica leaf extracts and recombinant yeast. *J. Plant Biol. 2007 503* **50**, 315–320 (2007).

17. Miettinen, K. *et al.* The seco-iridoid pathway from Catharanthus roseus. *Nat. Commun.* **5**, 1–12 (2014).

18. Qu, Y. *et al.* Solution of the multistep pathway for assembly of corynanthean, strychnos, iboga, and aspidosperma monoterpenoid indole alkaloids from 19E-geissoschizine. *Proc. Natl. Acad. Sci. U. S. A.* **115**, 3180–3185 (2018).

19. Caputi, L. *et al.* Missing enzymes in the biosynthesis of the anticancer drug vinblastine in Madagascar periwinkle. *Science (80-.).* **360**, 1235–1239 (2018).

20. Salim, V., Yu, F., Altarejos, J. & De Luca, V. Virus-induced gene silencing identifies Catharanthus roseus 7-deoxyloganic acid-7-hydroxylase, a step in iridoid and monoterpene indole alkaloid biosynthesis. *Plant J.* **76**, 754–765 (2013).

21. Asada, K. *et al.* A 7-Deoxyloganetic acid glucosyltransferase contributes a key step in secologanin biosynthesis in madagascar periwinkle. *Plant Cell* **25**, 4123–4134 (2013).

22. Qu, Y. *et al.* Completion of the seven-step pathway from tabersonine to the anticancer drug precursor vindoline and its assembly in yeast. *Proc. Natl. Acad. Sci. U. S. A.* **112**, 6224–6229 (2015).

23. Qu, Y. *et al.* Geissoschizine synthase controls flux in the formation of monoterpenoid indole alkaloids in a Catharanthus roseus mutant. *Planta* **247**, 625–634 (2018).

24. Tatsis, E. C. *et al.* A three enzyme system to generate the Strychnos alkaloid scaffold from a central biosynthetic intermediate. *Nat. Commun.* **8**, 1–10 (2017).

25. Lichman, B. R. *et al.* The evolutionary origins of the cat attractant nepetalactone in catnip. *Sci. Adv.* **6**, eaba0721 (2020).

26. Farrow, S. C. *et al.* Biosynthesis of an Anti-Addiction Agent from the Iboga Plant. *J. Am. Chem. Soc.* **141**, 12979–12983 (2019).

27. Schotte, C. *et al.* Directed Biosynthesis of Mitragynine Stereoisomers. *J. Am. Chem. Soc.* **145**, 4957–4963 (2023).

28. Croteau, R. Biosynthesis and Catabolism of Monoterpenoids. *Chem. Rev.* **87**, 929–954 (1987).

29. Bouwmeester, H. J., Gershenzon, J., Konings, M. C. J. M. J. M. & Croteau, R. Biosynthesis of the monoterpenes limonene and carvone in the fruit of caraway: I. Demonstration of enzyme activities and their changes with development. *Plant Physiol.* **117**, 901–912 (1998).

30. Iijima, Y., Gang, D. R., Fridman, E., Lewinsohn, E. & Pichersky, E. Characterization of Geraniol Synthase from the Peltate Glands of Sweet Basil. *Plant Physiol.* **134**, 370–379 (2004).

31. Takemoto, T., Nakajima, T., and Yokobe, T. & Takemoto Nakajima, T., and Yokobe, T., T. Structure of ibotenic acid. *J. Pharm. Soc. Japan* **84**, 1232–1233 (1964).

32. Geu-Flores, F. *et al.* An alternative route to cyclic terpenes by reductive cyclization in iridoid biosynthesis. *Nature* **492**, 138–142 (2012).

33. Lichman, B. R. *et al.* Uncoupled activation and cyclization in catmint reductive terpenoid biosynthesis. *Nat. Chem. Biol.* **15**, 71–79 (2019).

34. Murata, J., Roepke, J., Gordon, H. & De Luca, V. The leaf epidermome of Catharanthus roseus reveals its biochemical specialization. *Plant Cell* **20**, 524–542 (2008).

35. Guirimand, G. *et al.* The subcellular organization of strictosidine biosynthesis in Catharanthus roseus epidermis highlights several trans-tonoplast translocations of intermediate metabolites. *FEBS J.* **278**, 749–763 (2011).

36. Courdavault, V. *et al.* A look inside an alkaloid multisite plant: the Catharanthus logistics. *Curr. Opin. Plant Biol.* **19**, 43–50 (2014).

37. Larsen, B. *et al.* Identification of iridoid glucoside transporters in Catharanthus roseus. *Plant Cell Physiol.* **58**, 1507–1518 (2017).

38. Irmler, S. *et al.* Indole alkaloid biosynthesis in Catharanthus roseus: New enzyme activities and identification of cytochrome P450 CYP72A1 as secologanin synthase. *Plant J.* **24**, 797–804 (2000).

39. Bracher, D. & Kutchan, T. M. Strictosidine synthase from Rauvolfia serpentina: Analysis of a gene involved in indole alkaloid biosynthesis. *Arch. Biochem. Biophys.* **294**, 717–723 (1992).

40. Battersby, A. R. Biosynthesis—II. Terpenoid indole alkaloids. in *Alkaloids* vol. 1 31–47 (2007).

41. McCoy, E. & O'Connor, S. E. Directed Biosynthesis of Alkaloid Analogs in the Medicinal Plant Catharanthus roseus. *J. Am. Chem. Soc.* **128**, 14276–14277 (2006).

42. Bernhardt, P., McCoy, E. & O'Connor, S. E. Rapid Identification of Enzyme Variants for Reengineered Alkaloid Biosynthesis in Periwinkle. *Chem. Biol.* **14**, 888–897 (2007).

43. McCoy, E., Galan, M. C. & O'Connor, S. E. Substrate specificity of strictosidine synthase. *Bioorganic Med. Chem. Lett.* **16**, 2475–2478 (2006).

44. Runguphan, W., Qu, X. & O'Connor, S. E. Integrating carbon-halogen bond formation into medicinal plant metabolism. *Nature* **468**, 461–467 (2010).

45. Payne, R. M. E. *et al.* An NPF transporter exports a central monoterpene indole alkaloid intermediate from the vacuole. *Nat. Plants* **3**, 16208 (2017).

46. Luijendijk, T. J. C. C., Stevens, L. H. & Verpoorte, R. Purification and characterisation of strictosidine β-D-glucosidase from Catharanthus roseus cell suspension cultures. *Plant Physiol. Biochem.* **36**, 419–425 (1998).

47. Guirimand, G. *et al.* Strictosidine activation in Apocynaceae: Towards a 'nuclear time bomb'? *BMC Plant Biol.* **10**, 182 (2010).

48. Konno, K., Hirayama, C., Yasui, H. & Nakamura, M. Enzymatic activation of oleuropein: A protein crosslinker used as a chemical defense in the privet tree. *Proc. Natl. Acad. Sci. U. S. A.* **96**, 9159–9164 (1999).

49. Geerlings, A., Martinez-Lozano Ibañez, M., Memelink, J., Van Der Heijden, R. & Verpoorte, R. Molecular cloning and analysis of strictosidine $β$-D-glucosidase, an enzyme in terpenoid indole alkaloid biosynthesis in Catharanthus roseus. *J. Biol. Chem.* **275**, 3051–3056 (2000).

50. Gerasimenko, I., Sheludko, Y., Ma, X. & Stöckigt, J. Heterologous expression of a *Rauvolfia* cDNA encoding strictosidine glucosidase, a biosynthetic key to over 2000 monoterpenoid indole alkaloids. *Eur. J. Biochem.* **269**, 2204–2213 (2002).

51. Lobay, D. Rauvolfia in the Treatment of Hypertension. *Integr. Med.* **14**, 40–46 (2015).

52. Stavrinides, A. *et al.* Structural investigation of heteroyohimbine alkaloid synthesis reveals active site elements that control stereoselectivity. *Nat. Commun.* (2016) doi:10.1038/ncomms12116.

53. Flores-Bocanegra, L. *et al.* The Chemistry of Kratom [Mitragyna speciosa]: Updated Characterization Data and Methods to Elucidate Indole and Oxindole Alkaloids. *J. Nat. Prod.* **83**, 2165–2177 (2020).

54. Todd, D. A. *et al.* Chemical composition and biological effects of kratom (Mitragyna speciosa): In vitro studies with implications for efficacy and drug interactions. *Sci. Rep.* (2020) doi:10.1038/s41598-020-76119-w.

55. Takayama, H. Chemistry and pharmacology of analgesic indole alkaloids from the rubiaceous plant, Mitragyna speciosa. *Chemical and Pharmaceutical Bulletin* (2004) doi:10.1248/cpb.52.916.

56. Adams, A. M. *et al.* In vivo production of psilocybin in E. coli. *Metab. Eng.* **56**, 111–119 (2019).

57. Li, M. *et al.* Engineering a novel biosynthetic pathway in: Escherichia coli for the production of caffeine. *RSC Adv.* (2017) doi:10.1039/c7ra10986e.

58. Nakagawa, A. *et al.* A bacterial platform for fermentative production of plant alkaloids. *Nat. Commun.* **2**, 326 (2011).

59. Hausjell, J., Halbwirth, H. & Spadiut, O. Recombinant production of eukaryotic cytochrome P450s in microbial cell factories. *Biosci. Rep.* **38**, 20171290 (2018).

60. Luo, X. *et al.* Complete biosynthesis of cannabinoids and their unnatural analogues in yeast. *Nature* **567**, 123–126 (2019).

61. Galanie, S., Thodey, K., Trenchard, I. J., Interrante, M. F. & Smolke, C. D. Complete biosynthesis of opioids in yeast. *Science (80-.).* **349**, 1095–1100 (2015).

62. Kim, H., Yoo, S. J., Kang, H. A. & Alper, E. H. Yeast synthetic biology for the production of recombinant therapeutic proteins. *FEMS Yeast Res.* **15**, 1–16 (2015).

63. Roze, L. V., Chanda, A. & Linz, J. E. Compartmentalization and molecular traffic in secondary metabolism: A new understanding of established cellular processes. *Fungal Genetics and Biology* (2011) doi:10.1016/j.fgb.2010.05.006.

64. Dueber, J. E. *et al.* Synthetic protein scaffolds provide modular control over metabolic flux. *Nat. Biotechnol.* (2009) doi:10.1038/nbt.1557.

65. Hammer, S. K. & Avalos, J. L. Harnessing yeast organelles for metabolic engineering. *Nat. Chem. Biol.* **13**, 823–832 (2017).

66. Lin, J. L., Zhu, J. & Wheeldon, I. Synthetic Protein Scaffolds for Biosynthetic Pathway Colocalization on Lipid Droplet Membranes. *ACS Synth. Biol.* **6**, 1534–1544 (2017).

67. Ping, Y. *et al.* Building Microbial Hosts for Heterologous Production of N-Methylpyrrolinium. *ACS Synth. Biol.* (2019) doi:10.1021/acssynbio.8b00483.

68. Schwartz, C., Frogue, K., Misa, J. & Wheeldon, I. Host and pathway engineering for

enhanced lycopene biosynthesis in Yarrowia lipolytica. *Front. Microbiol.* **8**, (2017).

69. Milne, N. *et al.* Metabolic engineering of Saccharomyces cerevisiae for the de novo production of psilocybin and related tryptamine derivatives. *Metab. Eng.* **60**, 25–36 (2020).

70. Li, J., Feng, R., Wen, Z. & Zhang, A. Overexpression of ARO10 in pdc5Δmutant resulted in higher isobutanol titers in Saccharomyces cerevisiae. *Biotechnol. Bioprocess Eng.* **22**, 382–389 (2017).

71. Kent, R. & Dixon, N. Contemporary Tools for Regulating Gene Expression in Bacteria. *Trends in Biotechnology* vol. 38 316–333 (2020).

72. Trenchard, I. J. & Smolke, C. D. Engineering strategies for the fermentative production of plant alkaloids in yeast. *Metab. Eng.* **30**, 96–104 (2015).

73. Schwartz, C., Curtis, N., Löbs, A.-K. & Wheeldon, I. Multiplexed CRISPR Activation of Cryptic Sugar Metabolism Enables *Yarrowia Lipolytica* Growth on Cellobiose. *Biotechnol. J.* **1700584**, 1700584 (2018).

74. Schwartz, C., Frogue, K., Ramesh, A., Misa, J. & Wheeldon, I. CRISPRi repression of nonhomologous end-joining for enhanced genome engineering via homologous recombination in *Yarrowia lipolytica*. *Biotechnol. Bioeng.* **114**, 2896–2906 (2017).

75. Billingsley, J. M. *et al.* Engineering the biocatalytic selectivity of iridoid production in Saccharomyces cerevisiae. *Metab. Eng.* **44**, 117–125 (2017).

76. Harvey, C. J. B. *et al.* HEx: A heterologous expression platform for the discovery of fungal natural products. *Sci. Adv.* **4**, eaar5459 (2018).

77. Ro, D. K. *et al.* Production of the antimalarial drug precursor artemisinic acid in engineered yeast. *Nature* **440**, 940–943 (2006).

78. Li, M., Schneider, K., Kristensen, M., Borodina, I. & Nielsen, J. Engineering yeast for high-level production of stilbenoid antioxidants. *Sci. Rep.* **6**, (2016).

79. Hampton, R. Y. ER-associated degradation in protein quality control and cellular regulation. *Current Opinion in Cell Biology* vol. 14 476–482 (2002).

80. Sandig, G. *et al.* Regulation of endoplasmic reticulum biogenesis in response to cytochrome P450 overproduction. *Drug Metab. Rev.* **31**, 393–410 (1999).

81. Bond, C. M. & Tang, Y. Engineering Saccharomyces cerevisiae for production of simvastatin. *Metab. Eng.* **51**, 1–8 (2019).

82. Li, Y. & Smolke, C. D. Engineering biosynthesis of the anticancer alkaloid noscapine in yeast. *Nat. Commun.* **7**, 1–14 (2016).

83. Bat-Erdene, U. *et al.* Cell-Free Total Biosynthesis of Plant Terpene Natural Products Using an Orthogonal Cofactor Regeneration System. *ACS Catal.* **11**, 9898–9903 (2021).

84. Lee, S. M., Jellison, T. & Alper, H. S. Bioprospecting and evolving alternative xylose and arabinose pathway enzymes for use in Saccharomyces cerevisiae. *Appl. Microbiol. Biotechnol.* **100**, 2487–2498 (2016).

85. Srinivasan, P. & Smolke, C. D. Biosynthesis of medicinal tropane alkaloids in yeast.

Nature **585**, 614–619 (2020).

86. Wang, P., Wang, J., Zhao, G., Yan, X. & Zhou, Z. Systematic optimization of the yeast cell factory for sustainable and high efficiency production of bioactive ginsenoside compound K. *Synth. Syst. Biotechnol.* **6**, 69 (2021).

87. Fahy, J. Modifications in the upper or Velbenamine Part of the Vinca Alkaloids have Major Implications for Tubulin Interacting Activities. *Curr. Pharm. Des.* **7**, 1181–1197 (2001).

88. Latham, J., Brandenburger, E., Shepherd, S. A., Menon, B. R. K. & Micklefield, J. Development of Halogenase Enzymes for Use in Synthesis. *Chem. Rev.* **118**, 232–269 (2018).

89. Valliere, M. A., Korman, T. P., Arbing, M. A. & Bowie, J. U. A bio-inspired cell-free system for cannabinoid production from inexpensive inputs. *Nat. Chem. Biol.* (2020) doi:10.1038/s41589-020-0631-9.

90. Farrow, S. C. *et al.* Biosynthesis of an Anti-Addiction Agent from the Iboga Plant. *J. Am. Chem. Soc.* **141**, 12979–12983 (2019).

91. Caputi, L. *et al.* Missing enzymes in the biosynthesis of the anticancer drug vinblastine in Madagascar periwinkle. *Science (80-.).* **360**, 1235–1239 (2018).

92. Trenti, F. *et al.* Early and Late Steps of Quinine Biosynthesis. *Org. Lett.* **23**, 1793–1797 (2021).

93. Tanna, R. S. *et al.* Clinical Pharmacokinetic Assessment of Kratom (Mitragyna speciosa), a Botanical Product with Opioid-Like Effects, in Healthy Adult Participants. *Pharmaceutics* **14**, 620 (2022).

94. Gietz, R. D. & Schiestl, R. H. High-efficiency yeast transformation using the LiAc/SS carrier DNA/PEG method. *Nat. Protoc. 2007 21* **2**, 31–34 (2007).

95. Horwitz, A. A. *et al.* Efficient Multiplexed Integration of Synergistic Alleles and Metabolic Pathways in Yeasts via CRISPR-Cas. *Cell Syst.* **1**, 88–96 (2015).

96. Anthony, S. M. *et al.* Total Synthesis of (−)-Strictosidine and Interception of Aryne Natural Product Derivatives “Strictosidyne” and “Strictosamidyne”. *J. Am. Chem. Soc.* **143**, 7471–7479 (2021).