ENHANCED TRIACYLGLYCEROL PRODUCTION IN *Chlorella vulgaris* BY FLUX BALANCE ANALYSIS ON METABOLIC NETWORK

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A Thesis Submitted in Partial Fulfillment of the Requirements
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การเพิ่มการผลิตไตรเอซิลกลีเซอรอลใน *Chlorella vulgaris* โดยการวิเคราะห์สมดุลฟลักซ์บนโครงข่ายเมแทบอลิก

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วิทยานิพนธ์นี้เป็นส่วนหนึ่งของการศึกษาตามหลักสูตรปริญญาวิทยาศาสตรมหาบัณฑิต
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อิสรภาพ เขาทอง : การเพิ่มการผลิตไตรเอซิลกลีเซอรอลใน *Chlorella vulgaris* โดยการวิเคราะห์สมดุลฟลักซ์บนโครงข่ายเมแทบอลิก. (ENHANCED TRIACYLGLYCEROL PRODUCTION IN *Chlorella vulgaris* BY FLUX BALANCE ANALYSIS ON METABOLIC NETWORK) อ.ที่ปรึกษาหลัก : ผศ. ดร.กิติพร พลายมาศ, อ.ที่ปรึกษาร่วม : ผศ. ดร.อัญชิษฐา สัจจารักษ์

ไตรแอซิลไกลเซรอล (TAG) เป็นประเภทหนึ่งของไขมันที่สามารถถูกผลิตได้ใน สาหร่ายขนาดเล็ก ที่เรียกว่าคลอเรลลาวัลการิส (*Chlorella vulgaris*) ซึ่งเป็นแหล่งวัตถุดิบศักยภาพในการนำมาใช้ผลิตไบโอดีเซล อย่างไรก็ตามปัจจุบันการศึกษาวิจัยและแบบจำลองที่อธิบายถึงกระบวนการผลิตไขมันชนิดนี้โดยตรงยังมีไม่มากนัก ดังนั้นเพื่อที่จะแก้ไขปัญหานี้ จึงขอเสนอการใช้ เทคนิคเรียกว่า Linear minimization of metabolic adjustment (LMOMA) ที่สามารถทำนายผลของการแก้ไขยีนโดยอาศัยพื้นฐานของการวิเคราะห์สมดุลการไหลของกระแส (Flux balance analysis: FBA) เพื่อเพิ่มประสิทธิภาพในการผลิต TAG การทดลองการลบยีนเดียวในแบบจำลองทางเมตาบอลิกที่มีสเกลระดับจีโนมของโคเรลลาวัลริส สายพันธุ์ UTEX 395 (ชื่อย่อโมเดล iCZ843) ภายใต้เงื่อนไขการเจริญเติบโตสามรูปแบบที่แตกต่างกันได้แก่ เฮเทอโรโทรฟี (heterotrophy), โฟโตออโตโทรฟี (photoautotrophy) และ มิกโซโทรฟี (mixotrophy) ผลการศึกษาผบว่า การผลิต TAG เพิ่มขึ้น 5 เท่าและ 8 เท่าตามลำดับ ในสภาวะการเจริญแบบเฮเทอโรโทรฟีหลังลบยีน ‘genemark_Scaffold_1220-abinit-gene-0.13’ และ ‘genemark_Scaffold_1016-abinit-gene-0.16’ตามลำดับ นอกจากนี้ในสภาวะการเจริญแบบโฟโตออโตโทรฟี หลังจากการลบยีน ‘maker_Scaffold_332-augustus-gene-0.46’ พบการเพิ่มขึ้นของ TAG ที่ 7 เท่า ในขณะที่การลบยีนเดี่ยวในสภาวะการเจริญแบบมิกโซโทรฟีนั้น พบการเพิ่มผลผลิตของ TAG เพิ่มขึ้น 5 เท่า หลังลบยีน 'maker_Scaffold_33-augustus-gene-0.119' นอกจากนี้ ถึงแม้ว่าข้อมูลเกี่ยวกับการเกิดของวัฏจักรออกซิเดชันทุติยภูมิของกรดไขมัน (fatty acid β-oxidation) ในคลอเรลลาวัลการิสจะมีจำกัด งานวิจัยนี้ยังแสดงให้เห็นว่า การจัดการกระบวนการนี้อาจเป็นกลยุทธ์ที่มีความคุ้มค่าสำหรับการเพิ่มประสิทธิภาพในการผลิต TAG สำหรับสายพันธ์นี้ ดังนั้นการทำความเข้าใจและการจัดการกระบวนการเกิดของวัฏจักรออกซิเดชันทุติยภูมิของกรดไขมันของสายพันธุ์นี้นั้น สามารถช่วยเพิ่มพื้นฐานความรู้ในการพัฒนาสายพันธุ์ที่ให้ผลผลิต TAG ทีมีปริมาณได้สำหรับการใช้ในการผลิตเชื้อเพลิงชีวภาพ อาหาร และการใช้งานที่เป็นมิตรกับสิ่งแวดล้อม

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Triacylglycerol (TAG) is a type of lipid that can be produced by a microalga called *Chlorella vulgaris*, which is a promising source of biodiesel. However, there is a lack of research and models explaining how to directly produce this lipid. To address this, we propose using linear minimization of metabolic adjustment (LMOMA), which predicts gene manipulation effects based on Flux Balance Analysis (FBA), to enhance TAG production. We conducted single-gene deletion experiments on the genome-scale metabolic model of *C. vulgaris* UTEX 395 (iCZ843) under different growth conditions: heterotrophy, photoautotrophy, and mixotrophy conditions. We found that the TAG production increased by 5 and 8 times in heterotrophy after deleting genes ‘genemark_Scaffold_1220-abinit-gene-0.13’ and ‘genemark_Scaffold_1016-abinit-gene-0.16’, respectively. Moreover, in photoautotrophic, after the deletion of ‘maker_Scaffold_332-augustus-gene-0.46’ gene, we found that the TAG productions were increased at 7 times. In mixotrophic, the TAG production was increased at 5 times after the deletion of 'maker_Scaffold_33-augustus-gene-0.119'. Moreover, even if there is limited information about fatty acid β-oxidation in *Chlorella vulgaris*, our study suggests that manipulating this process could be a valuable strategy for enhancing TAG production for this species. Understanding and manipulating the fatty acid β-oxidation pathway could contribute to the development of high TAG strains for various applications, including biofuel, nutrition, and eco-friendly solutions.

Field of Study:	Bioinformatics and Computational Biology (Interdisciplinary Program)	Student's Signature .....
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CHAPTER 1 INTRODUCTION

1.1 BACKGROUND AND MOTIVATION

Biodiesel can be derived from animals and plants. This type of fuel is defined as renewable and environmentally friendly [1]. The other groups of organisms found to have potential for biofuel production are microalgae [2]. In microalgae, there are two major groups of lipids, polar lipids and non-polar lipids (neutral lipids). The polar lipids made up 41%-92% of total lipids while neutral lipids were approximately 5%-51% [3]. Triacylglycerol (TAG) is a neutral lipid that consists of three fatty acyl groups attached to the backbone called glycerol [4]. Among the lipid compounds, TAG is suitable for biodiesel production because TAG is favored over phospholipids or glycolipids in biofuel production due to their higher concentration of fatty acids and absence of phosphorus and sulfur. The presence of phosphate impedes the transesterification process, and research indicates that TAG exhibits a biodiesel yield of over 99% compared to phospholipids, which yield less than 70%[5], in contrast to other lipid compounds which are unlikely to produce biodiesel, but bio-oil with fuel properties. Since the lower degrees of unsaturation in lipids can directly impact the quality of biodiesel. It should be noted that the lower degrees of unsaturation in TAG are preferred for biodiesel production [6, 7]. Moreover, the more percentage of TAG in total lipids, the more quality of biodiesel gets [8]. During photosynthesis, TAG is accumulated in microalgal cells [9]. Unlike the glycerolipids found in membranes, TAG is not performed as a structural role but serves primarily as a storage form of carbon and energy. After being synthesized, TAG is deposited in densely packed lipid bodies located in the cytoplasm of the algal cell [10, 11].

TAG (also known as triglyceride) can be converted into biodiesel, fatty acid methyl ester, (also known as FAME) by trans-esterification with methanol [12]. In addition, the TAG pathway of plants and algae is identical based on Kennedy pathway [13].

Using microalgae as a feedstock offers several advantages as high reactivity or a high cetane number [14]. Additionally, it can be used as wastewater treatment while cultivating biodiesel [15] and so much more, which attracted for supplying the feedstock demands. The most remarkable microalga that has been used as a feedstock for biofuel is *Chlorella vulgaris*, primarily due to its high lipid and fatty acid content and ease of cultivation [16]. Under autotrophic state, the algal lipid content is approximately 25% of dry weight [17]. Furthermore, another study has been reported that for *C. vulgaris* a total lipid of which 41.3% was TAG.

Considering the low content of TAG in total lipid production in *C. vulgaris* to other algae, genetic manipulation should be performed to increase the algal TAG content. Several studies have been more focused on achieving a high amount of whole agal lipid contents but in reality, TAG is more favored in the biodiesel industry. Considering that, manipulation of genes involved in the production of the agal TAG will be beneficial for increasing the TAG production.

Conventional way to improve the commercial agal strain is mutagenesis. This time-consuming program is done by randomly screening for effective mutants (high production, for example) out of many survivors. Even if it is tedious and intensive working time, it is still used these days in industry since it comes off really well [18]. Another efficient way is using flux balance analysis (FBA), which is a tool that uses in the genome-scale of metabolism. FBA calculates the flow of metabolites throughout the metabolic network, which makes it possible to predict the growth rate and production rate of an organism [19]. Another function of FBA is analyzing the perturbed metabolic networks, which makes this tool suitable for predicting the results of gene manipulation [20-23]

There are several studies related to FBA, for example, (e.g., [24]) a flux-based approach was performed to analyze a fully compartmentalized metabolic of *C. vulgaris* (AG10032). They found that for this model (AG10032) a small amount of essential nutrients such as nitrate, as well as moderate levels of aeration, light intensity, and carbon dioxide can enhance the lipid content of this microalgae. Another example ([25]) was exploited genome-scale metabolic model of *Y.*

lipolytica (iYLl647) for metabolic engineering. The target production (dodecanedioic acid (DDDA)) was increased following the over-expression and deletion of a few reactions. All the reasons above make FBA an appropriate tool for studying and performing gene perturbation in one's organism.

Several previous studies in the field of biodiesel have focused on increasing the whole-cell lipid contents of *C. vulgaris*. However, in the process of producing biodiesel, only TAG can be used as a feedstock. The results from this study will contribute to the development of a more efficient TAG production system in *C. vulgaris* and help identify genes involved in FBA-based perturbations for the overproduction of TAG in *C. vulgaris*. This information will be valuable for genetic engineering efforts aiming to enhance the production of TAG in *C. vulgaris*. Additionally, this study can serve as a model for future research in this area.

1.2 RESEARCH OBJECTIVE

To identify the target genes for triacylglycerol (TAG) production enhancement by using flux balance analysis in *C. vulgaris*.

1.3 SCOPE OF THE RESEARCH

1.3.1 To assess the impact of single-gene deletion, resulting in high TAG content, on *C. vulgaris* UTEX395 (iCZ843).

1.3.2 To evaluate the effect of single-gene deletion strategy in each growth condition, including heterotrophic, photoautotrophic, and mixotrophic.

1.3.3 To evaluate the top genes that enhance TAG production in each growth condition, including heterotrophic, photoautotrophic, and mixotrophic.

1.3.4 To assess whether the gene manipulation prediction tools are suitable for the single-gene deletion strategy or not.

1.4 EXPECTED OUTCOMES

The obtained information from this study can be utilized to manipulate the genes in *C. vulgaris* and enhance TAG production.

CHAPTER 2 LITERATURE REVIEW

2.1 Biodiesel properties and quality

Biodiesel, a type of mono-alkyl ester, is derived from esters of fatty acid found in different oils and greases from plants and animals. To produce biodiesel, these oils, and greases need a process called transesterification. In the process the TAG reacts with alcohol, in the presence of a strong base, to form methyl ester, or biodiesel, and the by-product glycerol. Normally, biodiesel is generally a straight-chain fatty acid with 16 or 18 carbon atoms. However, its components can be varied due to the type of feedstock. For example, palmitic acid (16:0) is a versatile fatty acid that can be derived from various sources, including plants, animals, and microorganisms. Also, the contents of the palmitic acid in these organisms vary, for example, 20-30% of the animal total fatty acids and 10-40% of the plant total fatty acids [26]. Another common kind of fatty acid found in the feedstocks is oleic acid (18:1) as it is present as much as 70-80% of the total fatty acids of the olive oil [27]. Additionally, several nut oils also contain significant amounts of oleic acid [28]. In addition to these, there are other types of oil, e.g., lauric acid, myristic acid, and stearic acid as shown in Table 1.

Table 1 Common fatty acid group in biodiesel [29]

Common name	CAS no	Abbreviation Carbon atoms: Double bonds	Molecular formula	Molecular weight g/mol
Lauric acid	143–07-7	12:00	$C_{12}H_{24}O_2$	200.32
Myristic acid	544–63-8	14:00	$C_{14}H_{28}O_2$	228.38
Palmitic acid	57–10-3	16:00	$C_{16}H_{32}O_2$	256.43
Myristoleic acid	544–64-9	14:01	$C_{14}H_{26}O_2$	226.26
Palmitoleic acid	373–49-9	16:01	$C_{16}H_{30}O_2$	254.42
Stearic acid	57–11-4	18:00	$C_{18}H_{36}O_2$	284.48
Arachidic acid	506–30-9	20:00	$C_{20}H_{40}O_2$	312.54
Linoleic acid	60–33-3	18:02	$C_{18}H_{32}O_2$	280.46

Common name	CAS no	Abbreviation Carbon atoms: Double bonds	Molecular formula	Molecular weight g/mol
Linolenic acid	463–40-1	18:03	$C_{18}H_{30}O_2$	278.44
Oleic acid	112–80-1	18:01	$C_{18}H_{34}O_2$	282.47
Gondoic acid	5561–99-9	20:01	$C_{20}H_{38}O_2$	310.53
Erucic acid	112–86-7	22:01	$C_{22}H_{42}O_2$	338.58
Behenic acid	112–85-6	22:00	$C_{22}H_{44}O_2$	340.6

2.1.1 Biodiesel quality and properties

The fuel properties of biodiesel are influenced by various factors. These factors encompass the type and quality of the feedstock, composition of fatty acids, methods and parameters used in production, purification processes, as well as post-production considerations. Furthermore, biodiesel properties can be classified based on their effects on engine performance and operability. These include factors such as ignition quality, exhaust gas emissions, calorific value, specific fuel consumption, ease of operation, and combustion characteristics of the air-fuel mixture. The low-temperature properties of the fuel, such as cold filter plugging point (CFPP), cold soak filterability, cloud point, and pour point, are also important considerations [30]. Additionally, the important aspect to look for a good biodiesel is the cetane number, which tells the ignition properties of fuels. A high cetane number indicates better ignition quality[8], which is desirable for biodiesel, whereas ones with lower are not suitable for engine fuel[7]. Moreover, there are more criteria and testing methods to test the properties of biodiesel e.g., the American standards for testing materials (ASTM 6751-3) as described in Table 2 and the European Union (EN 14214) as described in Table 3. For instance, when considering the flash point, ASTM D6751-3 mandates a minimum flash point of 403K, whereas EN 14214 requires a minimum flash point of 101°C. Regarding sulfur content, ASTM D6751 sets a maximum sulfur

content of 0.05% by weight, while EN 14214 specifies a maximum sulfur content of 10mg per kg.

Table 2 American standards for testing materials (ASTM) standard for biodiesel and petro diesel fuel [31]

Property	Test method	ASTM D975 (petrodiesel)	ASTM D6751 (biodiesel, B100)
Flash point	D 93	325 K min	403 K
Water and sediment	D 2709	0.05 max %vol	0.05 max %vol
Kinematic viscosity (at 313 K)	D 445	1.3–4.1 mm2/s	1.9–6.0 mm2/s
Sulfated ash	D 874	–	0.02 max %wt
Ash	D 482	0.01 max %wt	–
Sulfur	D 5453	0.05 max %wt	–
Sulfur	D 2622/129	–	0.05 max %wt
Copper strip corrosion	D 130	No. 3 max	No. 3 max
Cetane number	D 613	40 min	47 min
Aromaticity	D 1319	35 max %vol	–
Carbon residue	D 4530	–	0.05 max %mass
Carbon residue	D 524	0.35 max %mass	–
Distillation temp (90% volume recycle)	D 1160	555 K min–611 K max	–

Table 3 European standard (EN 14214) for biodiesel [32]

Property	**Units**	**Lower limit**	**Upper limit**	**Test-method**
Ester content	% (m/m)	96.5	–	Pr EN 14103 d
Density at 15 °C	kg/m3	860	900	EN ISO 3675/EN ISO 12185
Viscosity at 40 °C	mm2/s	3.5	5	EN ISO 3104
Flash point	C	>101	–	ISO CD 3679e
Sulfur content	mg/kg	–	10	–
Tar remnant (at 10% distillation remnant)	% (m/m)	–	0.3	EN ISO 10370
Cetane number	–	51	–	EN ISO 5165
Sulfated ash content	% (m/m)	–	0.02	ISO 3987
Water content	mg/kg	–	500	EN ISO 12937
Total contamination	mg/kg	–	24	EN 12662

Property	Units	Lower limit	Upper limit	Test-method
Copper band corrosion (3 h at 50 °C)	rating	Class 1	Class 1	EN ISO 2160
Oxidation stability at 110 °C	h	6	–	pr EN 14112 k
Acid value	mg KOH/g	–	0.5	pr EN 14104
Iodine value	–	–	120	pr EN 14111
Linoleic acid methyl ester	% (m/m)	–	12	pr EN 14103d
Polyunsaturated (P4 double bonds) methylester	% (m/m)	–	1	–
Methanol content	% (m/m)	–	0.2	pr EN 141101
Monoglyceride content	% (m/m)	–	0.8	pr EN 14105m
Diglyceride content	% (m/m)	–	0.2	pr EN 14105m
Triglyceride content	% (m/m)	–	0.2	pr EN 14105m
Free glycerine	% (m/m)	–	0.02	pr EN 14105m/pr EN 14106
Total glycerine	% (m/m)	–	0.25	pr EN 14105m
Alkali metals (Na + K)	mg/kg	–	5	pr EN 14108/pr EN 14109
Phosphorus content	mg/kg	–	10	pr EN14107p

2.2 Biodiesel derived from microalgae

Microalgae, with an oil content of approximately 50%, have the potential to achieve biodiesel productivity of 86,515 kg per hectare per year. This value is much higher than that of soy crops and canola which yield approximately 170 and 340 kg. of biodiesel per hectare per year, respectively. The higher productivity of microalgae is due to their ability to produce a larger quantity of oil-rich biomass, making them an attractive feedstock for biodiesel production[33]. Certain species of microalgae, such as *Chlorella protothecoides*, *C. minutissima*, and *C. vulgaris*, can accumulate lipids up to significant proportions of their biomass. According to studies conducted by Wang, Li et al. (2008), Guarnieri, Nag et, al. (2013), and Espinosa-Gonzalez et al. (2014), these species have been found to accumulate lipids up to approximately 50%, 56%, and 60% of their dry weight, respectively. This high lipid content makes

these microalgal species particularly suitable for biodiesel production, especially *C. vulgaris* as it has high lipid content and is eco-friendly – able to grow in wastewater and has zero net CO_2 emission [36, 37].

2.2.1 Transesterification for Biodiesel production

Biodiesel production can be achieved from crude biodiesel through a chemical process called transesterification, wherein triglycerides react with alcohol in the presence of a catalyst (Figure 1). This reaction occurs in a series of three reversible steps, that convert triglycerides to diglycerides, diglycerides to monoglycerides, and monoglycerides to glycerol. Each step in this process results in the production of an ester, leading to the formation of three ester molecules from a single triglyceride molecule[38]. To facilitate the transesterification reaction, the catalyst such as sodium hydroxide and enzymes are generally utilized to break down the oil molecules by decreasing the elevated viscosity of TAG [39] .

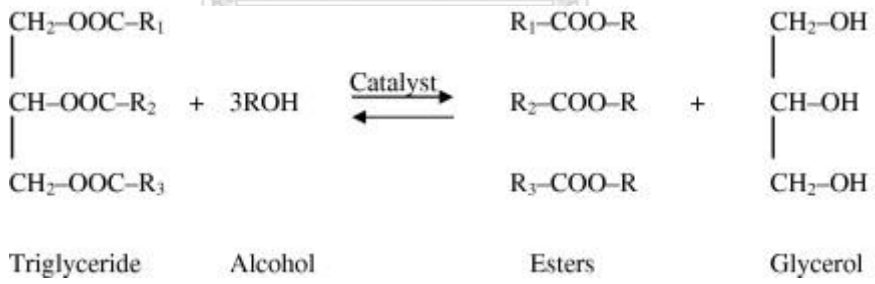

Figure 1 Transesterification of triacylglycerol with alcohol

2.2.2 TAG content of *C. vulgaris.*

According to 2.2 *C. vulgaris* attracted much attention not only for its spectacular lipid yields but also for its advantages as a feedstock for biodiesel production. This alga can produce TAG content of up to 41.3% of its total lipid contents [7] However, a study shows that it might be possible that the lipid content in this alga could be enhanced to 70% of its total lipid content

[40]. Therefore, unleashing the potential of the ratio between TAG and total lipids of this algal remains challenging.

2.2.3 Biosynthesis pathway of TAG in *C. vulgaris.*

In this study, the reference biosynthesis pathway of TAG in C. vulgaris was adapted from Parichehreh, Gheshlaghi et al. (2019) and Guarnieri, Nag et al. (2011) which were based on *C. vulgaris* and Chlorophyta and information from KEGG database [42]. As shown in Figure 2, TAG biosynthesis in *C. vulgaris* involves three steps: fatty acid synthesis, glycerol pathway, and TAG biosynthesis. In the first step – fatty acid synthesis – acetyl-CoA carboxylase (ACCase) catalyzes the conversion of acetyl-CoA to malonyl-CoA, where this activity of ACCase can be inhibited by AMP-activated kinase (AMPK) [41]. Malonyl-CoA is then transformed by malonyltransferase (MAT) into an acyl carrier protein (ACP), called malonyl-ACP. The condensation reaction takes place continuously with malonyl-ACP, resulting in the elongation of precursor acyl-ACP chains by two carbons per cycle, ultimately generating C16:0-ACP and C18:0-ACP. This condensation cycle is facilitated by 3-ketoacyl-acyl carrier protein synthase (KAS), beta-ketoacyl reductase (KAR), beta-hydroxyoctanoyl-acyl carrier protein dehydrase (HAD), and NADPH 2-enoyl Co A reductase (EAR). The final product of the cycle is then acted upon by fatty acyl-ACP thioesterase A (FAT). The resulting free fatty acids follow two paths – one leading to the cytosol and the other to TAG accumulation [43].

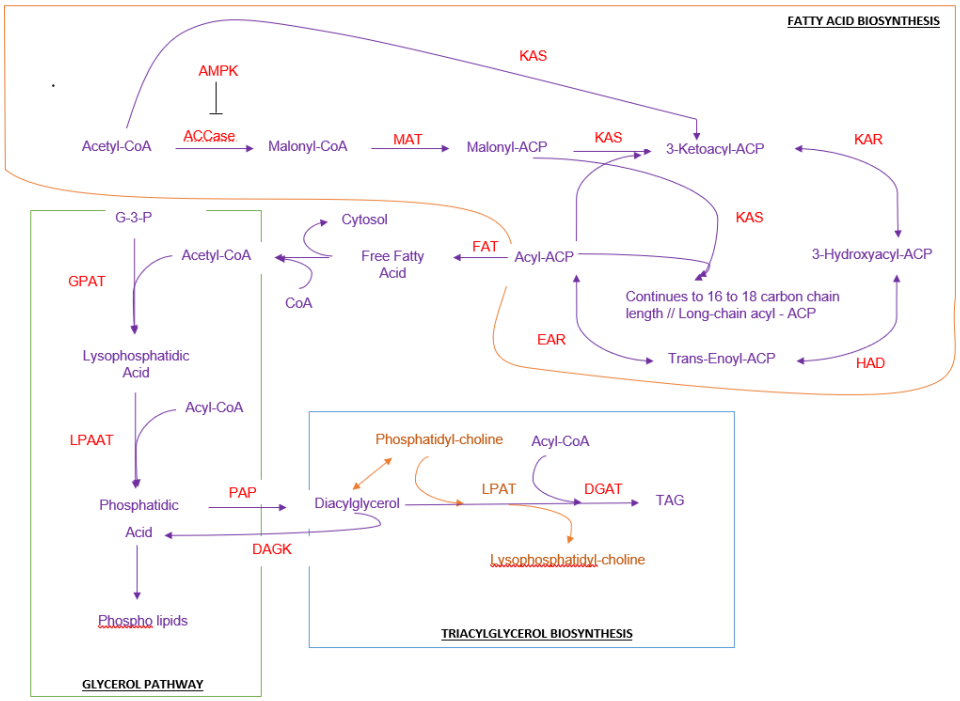

Figure 2 Biosynthesis pathway of triacylglycerol in Chlorella vulgaris*.*

Overview of TAG biosynthesize pathway in C. vulgaris*. The enzymes are indicated by red texts. The metabolites and pathway are indicated by black texts. The blunt arrows represent enzyme inhibitors. Ambiguous enzymes, which are not functionally certain, are in orange. ACCase, acetyl-CoA carboxylase; AMPK, AMP-activated kinase; MAT, malonyltransferase; KAS, 3-ketoacyl-acyl carrier protein synthase; KAR, beta-ketoacyl reductase; HAD, beta-hydroxyoctanoyl-acyl carrier protein dehydrase; EAR, NADPH 2-enoyl Co A reductase; FAT, fatty acyl-ACP thioesterase A; G-3-P, glyceraldehyde 3-phosphate; GPAT, glycerol 3-phosphate acyltransferase; LPAAT, 1-acylglycero-3-phosphate acyltransferase; PAP, phosphatic acid phosphatase; DAGK, diacylglycerol kinase (ATP); DAGT, 1,2-diacylglycerol acyltransferase; LPAT, lyso-phosphatidylcholine acyltransferase. Adapted from [24] and [41]based on* C. vulgaris *and Chlorophyta.*

As shown in Figure 2, the process of TAG biosynthesis involves the successive transfer of fatty acids from CoA to glycerol-3-phosphate (G3P) through the direct glycerol pathway [11, 44]. This sequential biosynthesis occurs through the catalytic action of

glycerol 3-phosphate acyltransferase (GPAT), which transfers acetyl-CoA to the first position of glyceraldehyde 3-phosphate (G-3-P), resulting in the production of lyso-phosphatidic acid (LPA). Subsequently, the acyl group is transferred to the second position of LPA through the action of 1-acylglycero-3-phosphate acyltransferase (LPAAT), forming phosphatic acid (PA). Additionally, PA can be formed via phosphorylation of diacylglycerol (DAG) catalyzed by diacylglycerol kinase (DAGK). [45]. Beginning of the penultimate step of TAG synthesis is start by the dephosphorylation of phosphatidic acid (PA) and the formation of DAG in the reaction catalyzed by phosphatic acid phosphatase (PAP). Consequently, a neutral TAG is formed in the final step by transferring the third acyl group to position three of DAG along with the catalyzing of 1,2-diacylglycerol acyltransferase (DAGT). Considering how the reactions involved in the TAG pathway affect the TAG accumulation in C. *vulgaris. The effect of single-gene deletion toward TAG production toward each condition of growth (heterotrophic, photoautotrophic, and mixotrophic) is considered worthwhile due to conditions of growth that can affect how production in microalgae should be. In photoautotrophic carbon dioxide and light are used to produce biomass for microalgae in the cultivation* [46]. Heterotrophic is culturing by providing high-cost carbon sources (normally glucose or acetate) without light. In mixotrophic culture, the carbon source is added along with the light as the main energy source [47]. In the study conducted by Sajadian, Morowvat et al. (2018), The impact of cultivation on C. vulgaris was studied, and it was observed that the heterotrophic condition resulted in the highest lipid contents (48.68%). These lipid contents were found to be 143.13% and 110.01% higher compared to the photoautotrophic (34.01%) and mixotrophic (44.25%) conditions, respectively. Moreover, the unique biological TAG accumulation in each compartment in *C. vugaris* was neglected, since the model per se calculated from the total TAG in the cytosol [48].

2.3 Genetic modification

Prior to the advent and application of molecular genetics in microbes, the predominant method for genetic modification involved the use of chemical or ultraviolet-induced mutagenesis. This technique aimed to induce mutations in the microbes' genome, followed by a subsequent enrichment or selection process to identify mutants exhibiting improved traits or characteristics, which can be quite labor-intensive and time-consuming. Another approach involves utilizing molecular genetics and a high level of precision of genetic engineering techniques in the laboratory to develop desired traits. This method has gained significant appeal and efficiency with the advancement of genomics and the accessibility of numerous fully sequenced microbial genomes to the public and thus is particularly favored by the industry [49].

2.3.1 Genetic manipulation prediction

Another effective tool for genetic manipulation is Flux Balance Analysis (FBA). This tool is one of the prediction tools used in genetic engineering. FBA is a mathematical approach used to predict the flux flow of reactions in a metabolic network as a linear programming model.

The flux balance equation can be represented by a stoichiometric matrix (S) of size *m* x *n*, where *m* stands for the number of metabolites and *n* stands for the number of reactions. V represents the vector of reaction fluxes as $\boldsymbol{v} = (\boldsymbol{v_1}, \ldots, \boldsymbol{v_n})$, Theoretically, for each intracellular metabolite the total of all fluxes producing the metabolite must equal to the total of all fluxes consuming the metabolite at a steady state in the system. Therefore, the linear equations that represent these flux balance constraints are

$$S \cdot \boldsymbol{v} = 0. \quad (1)$$

In reality, for a large-scale metabolic network model, there are more reactions (*n*) than metabolites (*m*), the system is undetermined and has an (*n*

- *m*) degree of freedom. This makes the system of equations cannot be solved algebraically. To solve the system, additional constraints would be supplied by setting the lower bounds and upper bounds of the reaction fluxes. These constraints define an allowable solution in space. Therefore, we obtain a linear programming model by maximizing an objective function as follows:

$$\text{Maximize /Minimize } Z = \boldsymbol{c}^T \boldsymbol{v} \qquad (2)$$

$$\text{Subject to } \boldsymbol{S}\boldsymbol{v} = 0$$

$$\boldsymbol{v}^{LB} \leq \boldsymbol{v} \leq \boldsymbol{v}^{UB},$$

where Z is an objective function. $\boldsymbol{c}$ is a vector of coefficients which determines the position of the reaction of interest as one; otherwise as zero. $\boldsymbol{v}$ is a reaction flux vector. $\boldsymbol{S}$ is a stoichiometric matrix. $\boldsymbol{v}^{LB}$ is a vector of lower bounds. $\boldsymbol{v}^{UB}$ is a vector of upper bounds.

2.3.2 Linear minimization of metabolic adjustment (FBA extension)

In general, we define an objective function as a biomass composition to contribute to the growth rate of an organism. To identify important genes or essential genes for an organism, we can simulate the way to delete a gene out of the model and detect the change in biomass production. However, the performance results of the FBA turn out as a non-accurate prediction of gene perturbation [50, 51]. Therefore, another approach called linear minimization of metabolic adjustment (or linear MOMA) is used to design a linear programing with an objective to minimize the change of the biomass after the gene deletion.

Linear MOMA is an extension of FBA used for simulating the effect of the perturbed gene. After FBA is performed, linear MOMA is used to solve the linear problem of the wild-type and mutant flux distributions. Because of the assumption that the optimality of a modified strain must remain as close as possible to the optimality of a wild-type strain. Notice that, the objective of

FBA is to maximize the biomass yield as mentioned in the equation above [52] while linear MOMA is to minimize the distance between v_{wt} and v_{del}. This can be simplified as the following.

$$min \sum |v_{wt-} v_{del}| \quad (3)$$

subject to

$$S_{wt} v_{wt} = 0,$$
$$c_{wt}^{T} v_{wt} = f_{wt},$$
$$S_{del} v_{del} = 0,$$
$$lb_{wt} \leq v_{wt} \leq ub_{wt}, \text{ and } lb_{del} \leq v_{del} \leq ub_{del},$$

where v_{wt} stands for reaction flux vector of wild type. v_{del} is the reaction flux vector of deletion (mutant). lb_{wt} is the lower bound of wild-type. lb_{del} is the lower bound of mutant. ub_{wt} is the upper bound of wild-type. ub_{del} is the upper bound of mutant. c_{wt}^{T} is the vector of coefficients which determines the position of the reaction of interest as one; otherwise as zero. The reaction of interest here belongs to wild-type. f_{wt} is the biomass, as an objective function in wild-type which in this study is defined as the linear sum of fluxes associated with the production of TAG. S_{wt} is the stoichiometric matrix belonging to the wild-type strain and S_{del} is the stoichiometric matrix belonging to the mutant strain.

2.4 Previous studies of achieving *C. vulgaris* strains with high TAG strains on *C. vulgaris* using genetic manipulation

Many genetic engineering studies have attempted to obtain a high lipid content in *C. vulgaris.* For instance, Norashikin et al. in 2018, discovered that overexpressing "**ω**-3- fatty acid desaturase" in *C. vulgaris* had an impact on the accumulation of higher **α**-linolenic acid (C18:3n3) content. Transformants grown of *C. vulgaris* in nitrogen-deficient conditions exhibited a noteworthy rise in total fatty acids (TFA), with levels increasing from 40% to 47% compared to the wild-type. Furthermore, compared to the wild type, the transformants demonstrated a slight

elevation in the proportion of α-linolenic acid within the TFA, with an increase from 8% to 10.8% [53].

Another study conducted by Lin and Ng in 2020 in *C. vulgaris* reported an increase in lipid accumulation at 46% after a plasmid containing Cas 9 fragment with sgRNA was constructed based on the gene for omega-3 fatty acid desaturase (fad3). However, considering that the high-quality biodiesel is made from TAG, not from other lipids, the remaining unsolved problem is how we can selectively increase only TAG production by *C. vulgaris* using genetic engineering prediction tools.

CHAPTER 3 EXPERIMENTAL

3.1 Genome-scale metabolic models of the *C. vulgaris*

The metabolic model utilized in this study is based on the reconstruction model of C. vulgaris UTEX 395, known as iCZ843. This model contained 843 genes, 2,294 reactions, 1,770 metabolites, and 6 compartments, including the cytoplasm, mitochondrion, chloroplast, thylakoid, glyoxysome, and extracellular space. The model consists of three conditions of growth namely heterotrophic, photoautotrophic, and mixotrophic. The model was constrained during the validation process using transcriptomics and other experimental data according to their respective conditions of growth[55].

3.1.1 Genome-scale metabolic model of *C. vulgaris* in the heterotrophic condition

A heterotrophic condition refers to a condition that algae are cultivated by feeding on organic carbon sources with no light. In this study, under the heterotrophic condition, *C. vulgaris* UTEX 395 was cultivated in silico in the medium and conditions as shown in Table 4.

Table 4 Heterotrophic medium and conditions [55].

Autotrophic-SBML File Properties	
File name	Cvu_hetero.xml
Organism	*Chlorella vulgaris* UTEX395
Model	*i*CVU843
Biomass Objective Function (BOF)	Biomass_Cvu_hetero-
Flux balance analysis objective	maximize BOF
Growth Associated Maintenance (GAM)	38.78 mmol ATP g_{DW}^{-1}
Non-Growth Associated Maintenance (NGAM)	1.5 mmol ATP g_{DW}^{-1} h^{-1}
Media conditions	Modified Bold's Basal Medium (MBBM)
Carbon source	Glucose 0.3025 mmol g_{DW}^{-1} h^{-1}
Aerobic or anaerobic	O_2 10 mmol g_{DW}^{-1} h^{-1}

Autotrophic-SBML File Properties	
Reactions constrained to zero	EX_photonVis(e), EX_ac(e), EX_glc-A(e), EX_co2(e), EX_starch(h), STARCH300DEGRA, STARCH300DEGRB, GLPThi, ATPSh, GAPDH(nadp)hi, MDH(nadp)hi, MDHC(nadp)hr, PPDKh, IDPh, PRUK, RBPCh, RBCh, SBP, H2Oth, Biomass_Cvu_mixo, Biomass_Cvu_photo

3.1.2 Genome-scale metabolic model of *C. vulgaris* in the phototrophic condition

A phototrophic condition is a condition where microalgae cultivate under light. In this study, under phototrophic condition, *C. vulgaris* UTEX 395 was cultivated in silico in the medium and conditions as shown in Table 5.

Table 5 Phototrophic medium and conditions [55].

Photoautotrophic-SBML File Properties	
File name	Cvu_photoauto.xml
Organism	*Chlorella vulgaris* UTEX395
Model	*i*CZ843
Biomass Objective Function (BOF)	Biomass_Cvu_photoauto-
Flux balance analysis objective	maximize BOF
Growth Associated Maintenance (GAM)	39.81 mmol ATP g_{DW}^{-1}
Non-Growth Associated Maintenance (NGAM)	1.5 mmol ATP g_{DW}^{-1} h^{-1}
Media conditions	Modified Bold's Basal Medium (MBBM)
Carbon source	CO_2 13.10 mmol g_{DW}^{-1} h^{-1}
Aerobic or anaerobic	O_2 10 mmol g_{DW}^{-1} h^{-1}
Photosynthetic oxygen evolution (DM_o2(u))	8.31 mmol g_{DW}^{-1} h^{-1}
Reactions constrained to zero	EX_ac(e), EX_glc-A(e), EX_starch(h), STARCH300DEGRB, STARCH300DEGRA, PCHLDR, G6PADHh, G6PBDHh, FBAh, H2Oth Biomass_Cvu_mixo, Biomass_Cvu_hetero

3.1.3 Genome-scale metabolic model of *C. vulgaris* in the mixotrophic condition

A mixotrophic condition refers to a situation in which microalgae grow in the presence of light along with organic and inorganic carbon-sources. In this study, under the mixotrophic condition, *C. vulgaris* UTEX 395 was cultivated in silico in the medium and conditions as shown in Table 6.

Table 6 Mixotrophic medium and conditions [55]

Autotrophic-SBML File Properties	
File name	Cvu_mix.xml
Organism	*Chlorella vulgaris* UTEX395
Model	*i*CVU843
Biomass Objective Function (BOF)	Biomass_cvu_mix-
Flux balance analysis objective	maximize BOF
Growth Associated Maintenance (GAM)	38.78 mmol ATP g_{DW}^{-1}
Non-Growth Associated Maintenance (NGAM)	1.5 mmol ATP g_{DW}^{-1} h^{-1}
Media conditions	Modified Bold's Basal Medium (MBBM)
Carbon source	CO_2 13.6 mmol g_{DW}^{-1} h^{-1}
	Glucose 0.3025 mmol g_{DW}^{-1} h^{-1}
Aerobic or anaerobic	O_2 10 mmol g_{DW}^{-1} h^{-1}
Photosynthetic oxygen evolution (DM_o2(u))	8.31 mmol g_{DW}^{-1} h^{-1}
Reactions constrained to zero	EX_photonVis(e), EX_ac(e), EX_glc-A(e), EX_co2(e), EX_starch(h), STARCH300DEGRA, STARCH300DEGRB, GLPThi, ATPSh, GAPDH(nadp)hi, MDH(nadp)hi, MDHC(nadp)hr, PPDKh, IDPh, PRUK, RBPCh, RBCh, SBP, H2Oth, Biomass_Cvu_photo-, Biomass_Cvu_hetero-

Moreover, environmental adaptability is a crucial characteristic observed in microalgae. Heterotrophic microalgae demonstrate a preference for low-light or dark environments, utilizing diverse organic compounds for their growth without relying on photosynthesis. Mixotrophic microalgae display remarkable adaptability by dynamically transitioning between autotrophic and heterotrophic modes depending on factors such as light intensity, nutrient availability, and the presence of organic carbon sources. In contrast, photoautotrophic microalgae heavily depend on

sufficient light intensity for photosynthesis, which is vital for their growth and productivity. It is important to note that microalgae strains within each category can exhibit considerable variations in their growth rates, nutritional requirements, and metabolic capabilities [56].

3.2 Bioinformatics Analysis

3.2.1 Model validation

The models used in this study were validated according to the method mentioned in Zuñiga, Li et al., 2016. Each model under three conditions of growth (heterotrophic, photoautotrophic, and mixotrophic) was used to predict the growth rate using flux balance analysis [19] in MATLAB R2021a. The predicted growth rates for heterotrophic (0.0168 $^{h-1}$), photoautotrophic (0.0248 $^{h-1}$), and mixotrophic (0.0402 $^{h-1}$). The predicted growth rates in each condition were concordant with the experimental data provided by Zuñiga, Li, et al., 2016 and Baalan, Pulich et al., 1973, which the predicted growth rates for heterotrophic (0.018-0.025 $^{h-1}$), photoautotrophic (0.014-0.025 $^{h-1}$) and mixotrophic (0.02-0.03 $^{h-1}$).

3.2.2 Analysis of single-gene deletion using Flux Balance Analysis (FBA) and Linear Minimization of Metabolic Adjustment (LMOMA)

This study was operated under MATLAB (R2021a) using COBRA Toolbox v.3.0 [58]. Moreover, each model of *C. vulgaris* UTEX 395 under three growth conditions, which were heterotrophic, photoautotrophic, and mixotrophic was used [55] as the models are up to date and suitable to examine any lipid productions. To achieve our goal the models of UTEX 395 were imported to MATLAB as .xml files. In the original model of each condition in .xml files the reactions that reach the production of TAG were screened out manually. All reactions involved in TAG production were imported into MATLAB and were assigned new vector positions (in this work assigned as 1) to distinguish the reactions involved in TAG production from others. After labeling the reactions involved in TAG production, the model was optimized using the

“optimizeCbModel” Function. This function is used to optimize a constraint-based model. Normally, the purpose of optimizing a constraint-based model is to determine the optimal values for the model's variables but this function was conducted to make sure that the labeling step didn’t make any change to the original models. Thereafter, all labeling fluxes with 1 were summed up to find the wild-type fluxes value toward TAG production in each condition of growth. Thenceforth, Import and operate the “SingleGeneDeletion(select ‘LMOMA’ for LMOMA and ‘FBA’ for FBA)” along with output [grRatio, grRateKO, grRateWT, hasEffect, delRxns, fluxSolution], This iterative function is deleting one gene at a time to see how it affect the whole model the result will be screen out for the gene that involved in the labeled reactions. The whole step of the predictive function using Flux balance analysis (FBA) and Linear Minimization of metabolic adjustment can be explained step by step, as shown in 3.2.1.1-3.2.1.8

3.2.2.1 Import the UTEX 395 model in MATLAB.

3.2.2.2 In the original model of UTEX 395 .xml, all of the reactions that reach to the production of TAG can be found here. Import all of the reactions involved in TAG production into MATLAB and assign vector positions in the model = 1.

3.2.2.3 Optimize the model using “optimizeCbModel” Function

3.2.2.4 Calculate all flux values of (3.2.1.2) in the model (only fluxes with vector position 1 were calculated)

3.2.2.5 Import and operate “SingleGeneDeletion(select ‘LMOMA’ for LMOMA and ‘FBA’ for FBA)” along with output [grRatio, grRateKO, grRateWT, hasEffect, delRxns, fluxSolution]

3.2.2.6 Calculate the total flux value of each (2.2) after Single-gene deletion

3.2.2.7 Screen out the flux values (2.6) that have undergone Single-gene deletion in which the results are higher than in wild-type (wild-type flux = 0.0168 mmol g^{-1}dry weight h^{-1})

3.2.2.8 Find genes in (2.7) by using the “model.genes” function

3.3 Identification of important knocked-out genes whose mutants cause high production of TAG

To identify knocked-out genes that reach the enhancement of TAG, by performing the single gene deletion with both the conventional method (FBA) and the derived method based on FBA, known as linear MOMA. Knocked-out genes can be identified by comparing the total flux sum of the mutant with that of the wild-type. If the ratio of the comparison is greater than one, it means this mutant produces more TAG than the wild-type. In other words, if the ratio of the comparison is less than one, it means this mutant produces less TAG than the wild-type. If the ratio is close to zero, the mutant cannot produce any compound of interest.

The results of the single-gene deletion were then evaluated with KEGG by manually checking their respective pathway against pathways found in KEGG to identify biological pathways for each gene list. Moreover, each targeted gene was then chosen for the top five genes to represent each condition of growth along with the results of other literature. Then, the resulting genes were used to perform BLASTX analysis against NCBI non-redundant protein database [59], Analysis of the single gene deletion approach using the FBA method in heterotrophic condition resulted in 30 targeted genes that could increase the agal TAG production (Table 7). However, these results did not seem to reflect the algal biological adaptation as all the single-gene deletions resulted in the same flux value decrease, all of them showed the same flux value (0.001824679 mmol g^{-1}dry weight h^{-1}) which was lower than the flux wild-type (0.0168 mmol g^{-1}dry weight h^{-1}). Thus, the FBA method was not suitable for the prediction of single-gene deletion.

CHAPTER 4 RESULTS AND DISCUSSION

In this study, I evaluated the effect of single-gene deletion on three growth conditions (heterotrophic, phototrophic, and mixotrophic) of *C. vulgaris* to obtain mutants with a high TAG production using FBA and LMOMA methods. Results suggested that deletion of one gene in *C. vulgaris* growing in silico under these growth conditions could increase the algal TAG production. In addition, the LMOMA method was more suitable than the FBA method for these algal models.

4.1 Identification of targeted genes in each condition of growth using FBA

Analysis of the single gene deletion approach using the FBA method in heterotrophic condition resulted in 30 targeted genes that could increase the agal TAG production (Table 7). However, these results did not seem to reflect the algal biological adaptation as all the single-gene deletions resulted in the same flux value decrease, all of them showed the same flux value (0.001824679 mmol g^{-1}dry weight h^{-1}) which was lower than the flux wild-type (0.0168 mmol g^{-1}dry weight h^{-1}). Thus, the FBA method was not suitable for the prediction of single-gene deletion.

Table 7 Flux value after single gene deletion using FBA, heterotrophy

Gene	Flux value
'maker_Scaffold_313-snap-gene-0.153'	0.001824679
'maker_Scaffold_134-augustus-gene-0.162'	0.001824679
'maker_Scaffold_141-augustus-gene-0.146'	999.1440103
'genemark_Scaffold_458-abinit-gene-0.18'	0.001824679
'maker_Scaffold_225-augustus-gene-0.195'	0.001824679
'maker_Scaffold_591-augustus-gene-0.57'	0.001824679
'maker_Scaffold_1694-augustus-gene-0.45'	0.001824679
'maker_Scaffold_1358-augustus-gene-0.13'	0.001824679
'maker_Scaffold_134-augustus-gene-0.159'	0.001824681
'maker_Scaffold_13-augustus-gene-0.157'	0.001824681
'maker_Scaffold_1839-snap-gene-0.18'	0.001824679
'augustus_masked_Scaffold_13-abinit-gene-0.17'	0.001824679
'maker_Scaffold_732-augustus-gene-0.46'	0.001824679
'maker_Scaffold_224-augustus-gene-0.64'	0.001824679
'genemark_Scaffold_37-abinit-gene-0.8'	0.001824679

Gene	Flux value
'maker_Scaffold_2346-augustus-gene-0.12'	0.001824679
'maker_Scaffold_214-augustus-gene-0.266'	0.001824679
'maker_Scaffold_439-snap-gene-0.203'	0.001824679
'maker_Scaffold_363-augustus-gene-0.154'	0.001824679
'maker_Scaffold_1137-snap-gene-0.128'	0.001824679
'maker_Scaffold_145-augustus-gene-0.53'	0.001824679
'maker_Scaffold_119-augustus-gene-0.91'	0.001824679
'maker_Scaffold_374-augustus-gene-0.178'	0.001824679
'maker_Scaffold_2109-augustus-gene-0.10'	0.001824679
'genemark_Scaffold_704-abinit-gene-0.31'	0.001824679
'maker_Scaffold_2053-augustus-gene-0.13'	0.001824679
'genemark_Scaffold_1331-abinit-gene-0.9'	0.001824679
'maker_Scaffold_431-augustus-gene-0.24'	0.001824679
'maker_Scaffold_395-augustus-gene-0.52'	0.001824679
'maker_Scaffold_895-snap-gene-0.40'	0.001824679

4.2 Identification of targeted genes in heterotrophic, photoautotrophic, and mixotrophic, conditions using LMOMA.

To identify target genes, firstly, the sum flux values of TAG in *C. vulgaris,* before gene deletion, growing in heterotrophic, mixotrophic, and photoautotrophic conditions were examined. The results showed that the sum flux values were 0.0018, 0.0043, and 0.0020 mmol g^{-1}dry weight h^{-1}, respectively. Then, the flux values in each condition were recalculated after single-gene deletions. Genes were only selected if their deletion resulted in a higher sum flux value of TAG.

4.2.1 Desirable mutants from the simulation of single-gene deletions in the heterotrophic condition

For heterotrophic condition, single-gene deletion of 55 genes resulted in a higher sum flux value (Table 8 and APPENDIX A). The top five genes and their respective functions were genemark_Scaffold_1016-abinit-gene-0.16 (cofactor recycling subsystem), maker_Scaffold_439-snap-gene-0.203 (glycine, serine, and threonine metabolism subsystem and valine, leucine and

isoleucine biosynthesis subsystem), maker_Scaffold_145-augustus-gene-0.53 (glyoxylate and dicarboxylate metabolism subsystem), genemark_Scaffold_1220-abinit-gene-0.13 (fatty acid metabolism, propanoate metabolism, and alpha-linolenic acid metabolism subsystem) and maker_Scaffold_1153-augustus-gene-0.48 (starch metabolism).

The first gene, genemark_Scaffold_1220-abinit-gene-0.13, was involved in fatty acid metabolism, propanoate metabolism, and alpha-linolenic acid metabolism. This reaction in plants and humans involved in beta-oxidation results in hormone and enzyme productions, this beta-oxidation decreases the amount of TAG by degrading it to produce acetyl-CoA molecules [60, 61]. Moreover, acetyl-CoA was generated after the degradation of fatty acid. Will be involved in the generation of TAG [62],[63] explaining by, after the degradation of free fatty acid in the glycerol pathway. Acetyl-CoA which involved in the generation of phosphatidic acid. Phosphatidic acid is involved in the initial stage of TAG production in TAG biosynthesis as shown in Figure 2., which steps contribute to the decrease in total TAG in a cell.

Another interesting sequence was genemark_Scaffold_1016-abinit-gene-0.16, encoding for an electron transfer flavoprotein-ubiquinone oxidoreductase (ETF-QO), this reaction is known as a short electron transfer pathway. ETF-QO transfers electrons from nine different mitochondrial FAD-containing amino acids metabolism and acyl-CoA dehydrogenases of fatty acid beta-oxidation to the ubiquinone pool of the main respiratory chain[64]. ETF-QO system can alter the changes of TAG since the system contains acyl-CoA dehydrogenases of fatty acid beta-oxidation. This can be explained by, after the hydrolysis of TAG, fatty acids as products will undergo beta-oxidation resulting in the product of acetyl-CoA. Then the acetyl-CoA will be used in the mitochondrial respiratory chain together with electron transfer

flavoprotein(ETF) [65], this ETF-QO system alter the total TAG by breaking it to produce acetyl-CoA production resulting in the lowering of TAG production.

In congruence with the reactions involved by the resulted gene sequences. Our blastx results suggested that these five genes could putatively be the FMN-linked oxidoreductases superfamily protein, threonine dehydratase, glycerate dehydrogenase, acyl-coenzyme A oxidase peroxisomal, and beta-amylase (APPENDIX G), which also support the reason why deletion of these genes might enhance TAG production of *C. vulgaris*.

Hence, we conclude that deleting the gene involved in utilizing acetyl-CoA to other products is an effective strategy in heterotrophic condition, which in this study shows in rising of TAG 5 times after the deletion of genemark_Scaffold_1220-abinit-gene-0.13. The highest results show to have the TAG at 8.061 times after the deletion of (genemark_Scaffold_1016-abinit-gene-0.16).

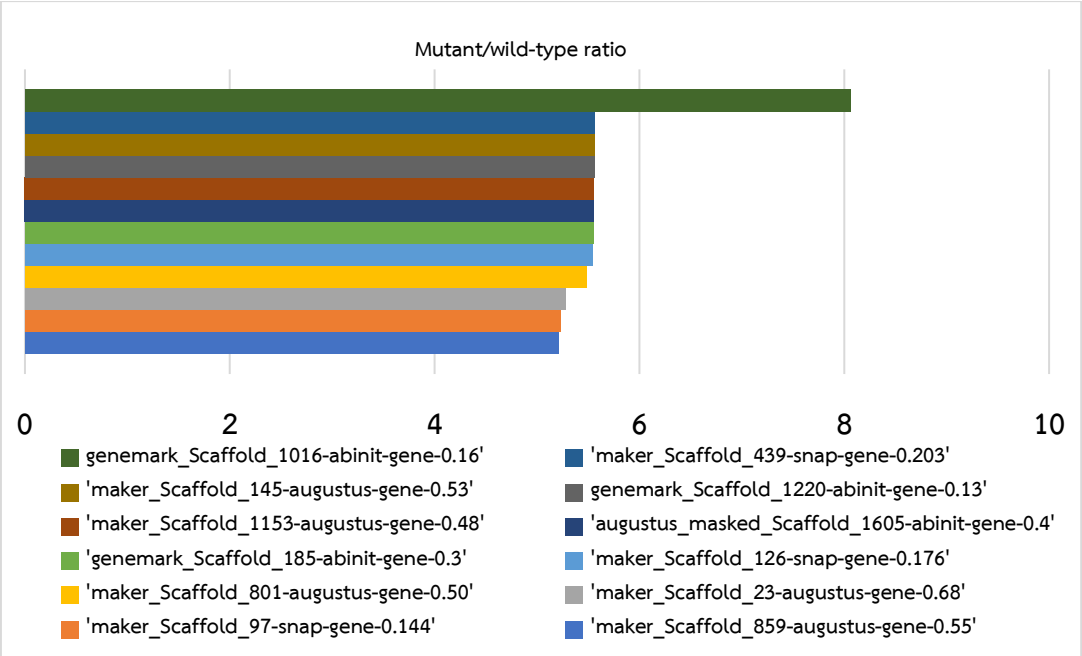

Figure 3 Mutant/wild-type ratios for top twelve potential knocked-out genes as their mutants yield higher than fivefold compared to the wild-type (heterotrophy).

Table 8 Mutant/wildtype ratio under heterotrophic condition

Gene List	Mutant/wildtype ratio
'genemark_Scaffold_1016-abinit-gene-0.16'	8.061394724
'maker_Scaffold_439-snap-gene-0.203'	5.557899113
'maker_Scaffold_145-augustus-gene-0.53'	5.557898894
'genemark_Scaffold_1220-abinit-gene-0.13'	5.557864857
'maker_Scaffold_1153-augustus-gene-0.48'	5.556460194
'augustus_masked_Scaffold_1605-abinit-gene-0.4'	5.556460194
'genemark_Scaffold_185-abinit-gene-0.3'	5.553627655
'maker_Scaffold_126-snap-gene-0.176'	5.537859341
'maker_Scaffold_801-augustus-gene-0.50'	5.487958113
'maker_Scaffold_23-augustus-gene-0.68'	5.273919978
'maker_Scaffold_97-snap-gene-0.144'	5.232032156
'maker_Scaffold_859-augustus-gene-0.55'	5.207149276
'maker_Scaffold_1306-snap-gene-0.47'	5.196585076
'genemark_Scaffold_484-abinit-gene-0.7'	5.110905501

Gene List	Mutant/wildtype ratio
'maker_Scaffold_176-augustus-gene-0.194'	5.110905501
'genemark_Scaffold_211-abinit-gene-0.14'	4.67966903
'maker_Scaffold_500-augustus-gene-0.34'	4.449639908
'maker_Scaffold_969-augustus-gene-0.37'	4.316446334
'maker_Scaffold_458-augustus-gene-0.118'	4.252969986
'maker_Scaffold_313-snap-gene-0.153'	3.717485638
'maker_Scaffold_1851-snap-gene-0.13'	3.717464849
'augustus_masked_Scaffold_835-abinit-gene-0.5'	3.391603054
'maker_Scaffold_556-augustus-gene-0.46'	3.391603054
'maker_Scaffold_899-augustus-gene-0.126'	3.391603054
'genemark_Scaffold_708-abinit-gene-0.19'	3.391603054
'maker_Scaffold_2123-augustus-gene-0.11'	3.352731226
'maker_Scaffold_137-augustus-gene-0.65'	3.352731226
'maker_Scaffold_1615-augustus-gene-0.55'	3.352731226
'maker_Scaffold_119-augustus-gene-0.94'	3.352731226
'maker_Scaffold_758-augustus-gene-0.38'	3.352731226
'augustus_masked_Scaffold_79-abinit-gene-0.2'	3.32151061
'maker_Scaffold_329-augustus-gene-0.180'	3.146733379
'GL433847.1.100'	2.924263731
'maker_Scaffold_645-augustus-gene-0.44'	2.844072255
'maker_Scaffold_21-augustus-gene-0.94'	2.8427507
'maker_Scaffold_93-augustus-gene-0.49'	1.818296335
'maker_Scaffold_141-augustus-gene-0.146'	1.001576807
'maker_Scaffold_772-augustus-gene-0.100'	1.000499218
'augustus_masked_Scaffold_755-abinit-gene-0.7'	1.00000027
'maker_Scaffold_208-augustus-gene-0.149'	1.000000067
'snap_masked_Scaffold_556-abinit-gene-0.35'	1.000000066
'maker_Scaffold_916-snap-gene-0.4'	1.000000059
'maker_Scaffold_1137-snap-gene-0.128'	1.00000002
'maker_Scaffold_374-augustus-gene-0.178'	1.000000012

Gene List	Mutant/wildtype ratio
'maker_Scaffold_1358-augustus-gene-0.13'	1.000000007
'genemark_Scaffold_366-abinit-gene-0.45'	1.000000006
'maker_Scaffold_504-augustus-gene-0.24'	1.000000006
'genemark_Scaffold_37-abinit-gene-0.8'	1.000000001
'snap_masked_Scaffold_291-abinit-gene-0.6'	1.000000001
'augustus_masked_Scaffold_1130-abinit-gene-0.2'	1.000000001
'genemark_Scaffold_1087-abinit-gene-0.4'	1.000000001
'maker_Scaffold_1038-augustus-gene-0.43'	1.000000001
'maker_Scaffold_156-augustus-gene-0.44'	1.000000001
'maker_Scaffold_685-augustus-gene-0.113'	1.000000001
'maker_Scaffold_2053-augustus-gene-0.13'	1.000000001

4.2.2 Desirable mutants from the simulation of single-gene deletions in the phototrophic condition

For the phototrophic condition, single-gene deletion of 11 genes resulted in a higher sum flux value (Table 9 and APPENDIX B). The top eight genes and their respective functions were maker_Scaffold_332-augustus-gene-0.46 (galactose metabolism subsystem), genemark_Scaffold_704-abinit-gene-0.31 (purine metabolism, and pyrimidine metabolism subsystem), augustus_masked_Scaffold_13-abinit-gene-0.17' (cysteine, methionine, purine, pyrimidine, and selenoamino acid metabolism subsystem), maker_Scaffold_1851-snap-gene-0.13 (transport, extracellular, ascorbate, and aldarate metabolism subsystem) and genemark_Scaffold_2835-abinit-gene-0.1 (pyrimidine metabolism).

The first gene, maker_Scaffold_332-augustus-gene-0.46 (7.475 times higher in TAG production) is involved in galactose metabolism. Concordantly, in 2017 [66]studied green algae model (*Chlamydomonas reinhardtii*) and found that in photosynthesis or glycolysis, excessive carbon was obtained and

subsequently re-distributed into carbon-containing compounds, and then diverted into lipid metabolism on the way to produce storage lipids via the gamma-aminobutyrate pathway, glycolysis, and the Krebs' cycle. Moreover, the study shows that the products of glycolysis such as glucose-6-phosphate, phosphoenolpyruvate, and leucine influence lipid metabolism. These excess carbon-containing compounds play roles in distributing excess carbon for carbohydrate and lipid metabolism. Many sugar molecules such as glucose, glucoheptose, galactose, fructose, raffinose, ribose, ribulose, maltotetrarose, mannose, arabinose, and N-acetylglucosamine were diverted into carbohydrate storage molecules. In addition, a decrease in citrate and excess carbon flow from the Krebs' cycle was transferred to lipid metabolism [67]. Hence, this forces the carbon to enter the lipid metabolism to form TAG resulting in a higher rate of TAG in this species under photoautotrophic conditions.

Another interesting sequence was genemark_Scaffold_704-abinit-gene-0.31, and augustus_masked_Scaffold_13-abinit-gene-0.17 After the deletion of these single genes, the production of TAG increased by 7.475 times in both cases. According to the study by Chen in 2017, indicated that the nitrogen assimilation and distribution pathways related to nucleic acid metabolism are not the major pathway contributed to the lipid metabolic pathway. Thus, in photoautotrophic conditions, carbon assimilation should be focused as one of strategy.

In congruence with the reactions involved by the resulting gene sequences. Our blastx results suggested that these five genes could putatively be the inositol phosphorylceramide glucuronosyltransferase 1, ribonucleoside-diphosphate reductase small chain, NADPH-protochlorophyllide oxidoreductase, copper-transporting ATPase, and aspartate carbamoyltransferase chloroplastic (APPENDIX H), which also

support the reason why deletion of these genes might enhance TAG production of *C. vulgaris*.

Moreover, it is worthwhile to explore more on carbon and nitrogen assimilation toward the production of TAG. In our study, the numbers of TAG production after single-gene deletion toward gene involved in carbon and nitrogen assimilation share nearly the same amount of production (7.4752 times, 7.4751 times and 7.451 times in maker_Scaffold_332-augustus-gene-0.46, genemark_Scaffold_704-abinit-gene-0.31, and augustus_masked_Scaffold_13-abinit-gene-0.17 respectively).

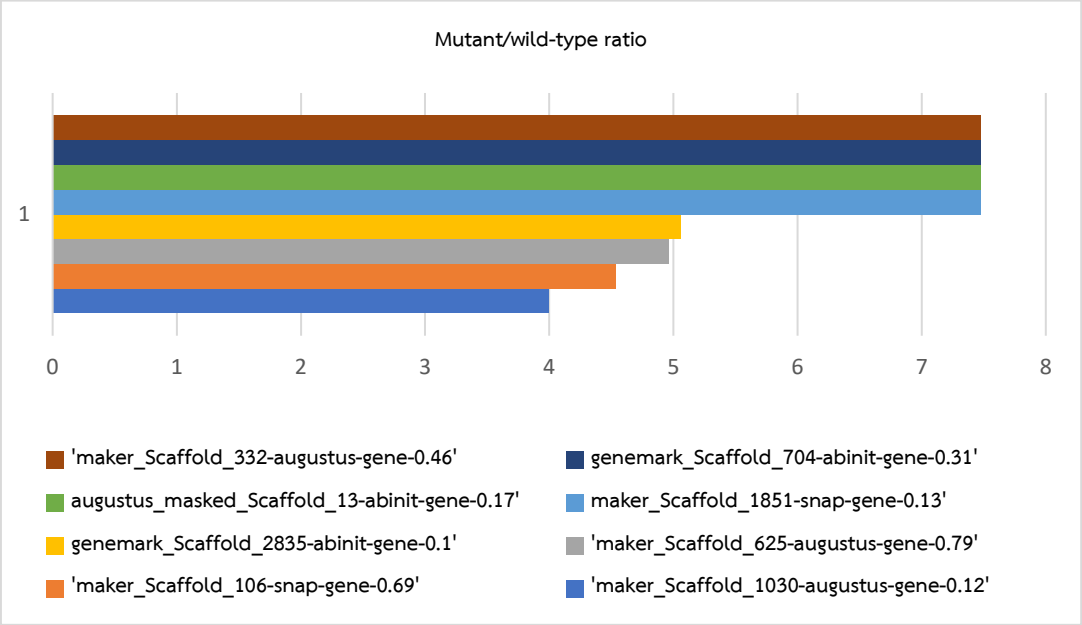

Figure 4 Mutant/wild-type ratios for top eight potential knocked-out genes as their mutants yield higher than three-fold compare to the wild-type (photoautrophy).

Table 9 Mutant/wild-type ratio under photoautrophic condition

Gene List	Mutant/wildtype ratio
'maker_Scaffold_332-augustus-gene-0.46'	7.475294396
'maker_Scaffold_1851-snap-gene-0.13'	7.475138706
'augustus_masked_Scaffold_13-abinit-gene-0.17'	7.475138706
'genemark_Scaffold_704-abinit-gene-0.31'	7.475138706
'genemark_Scaffold_2835-abinit-gene-0.1'	5.0595272
'maker_Scaffold_625-augustus-gene-0.79'	4.962347583

Gene List	Mutant/wildtype ratio
'maker_Scaffold_106-snap-gene-0.69'	4.53302595
'maker_Scaffold_1030-augustus-gene-0.12'	3.993345964
'genemark_Scaffold_335-abinit-gene-0.8'	1.120085158
'maker_Scaffold_1801-augustus-gene-0.22'	1.120085158
'genemark_Scaffold_651-abinit-gene-0.33'	1.023273216

4.2.3 Desirable mutants from the simulation of single-gene deletions in mixotrophic condition

For the mixotrophic condition, single-gene deletion of 10 genes resulted in a higher sum flux value (Table 10 and APPENDIX C). The top nine genes and their respective functions were maker_Scaffold_33-augustus-gene-0.119 (TCA cycle; carbon fixation; CO2 fixation), maker_Scaffold_1801-augustus-gene-0.22 (fatty acid metabolism and glycerolipid metabolism subsystem), maker_Scaffold_106-snap-gene-0.69 (carbon fixation subsystem), genemark_Scaffold_1057-abinit-gene-0.17 (transport, chloroplast and, transport, thylakoid lumen subsystem), and maker_Scaffold_308-augustus-gene-0.94 (carotenoid biosynthesis).

The first gene, genemaker_Scaffold_33-augustus-gene-0.119; showed an increase of TAG at 5.557 times, which is involved in the TCA cycle; carbon fixation; CO2 fixation subsystem, specifically in the malate dehydrogenase (NAD). For NAD is involved in energetic exchanges that occur from peroxisome to chloroplast together with redox-based signaling [68]. The energetic interactions between chloroplast and peroxisome were little-known till these days, even though these two organelles are often spotted and located close to one another [69, 70]. Inter-organelle communication between these two organelles is important to maintain chloroplast redox poise. The chloroplast is excessively reduced in its absence causing the activation of fatty acid and starch synthesis [71]. According to the study conducted by Kong and colleagues in 2018, it was found that knocking out malate dehydrogenase 2

can lead to a significant increase of 50% in lipid content. This increase is attributed to the blocking of the malate dehydrogenase step, which subsequently results in the inhibition of fatty acid beta-oxidation.

Another interesting sequence was maker_Scaffold_1801-augustus-gene-0.22; at 4.571 times TAG production. In which involved in fatty acid metabolism and glycerolipid metabolism subsystem, which is directly relevant to TAG production, see more details in APPENDIX F.

In congruence with the reactions involved by the resulting gene sequences. Our blastx results suggested that these five genes could putatively be the transmembrane 9 superfamily member 3, hypothetical protein D9Q98_003261, lycopene beta chloroplastic, and 3-hydroxybutyryl-dehydrogenase, which also support the reason why deletion of these genes might enhance TAG production of *C. vulgaris*.

Based on all the evidence presented, for mixotrophic genetic engineering toward a gene related to NAD; maker_Scaffold_33-augustus-gene-0.119 seems to be the effective way to rate up the TAG production with up to 5-fold of the original production, but more research is needed for a clear explanation of how energetic interactions between chloroplast and peroxisome works.

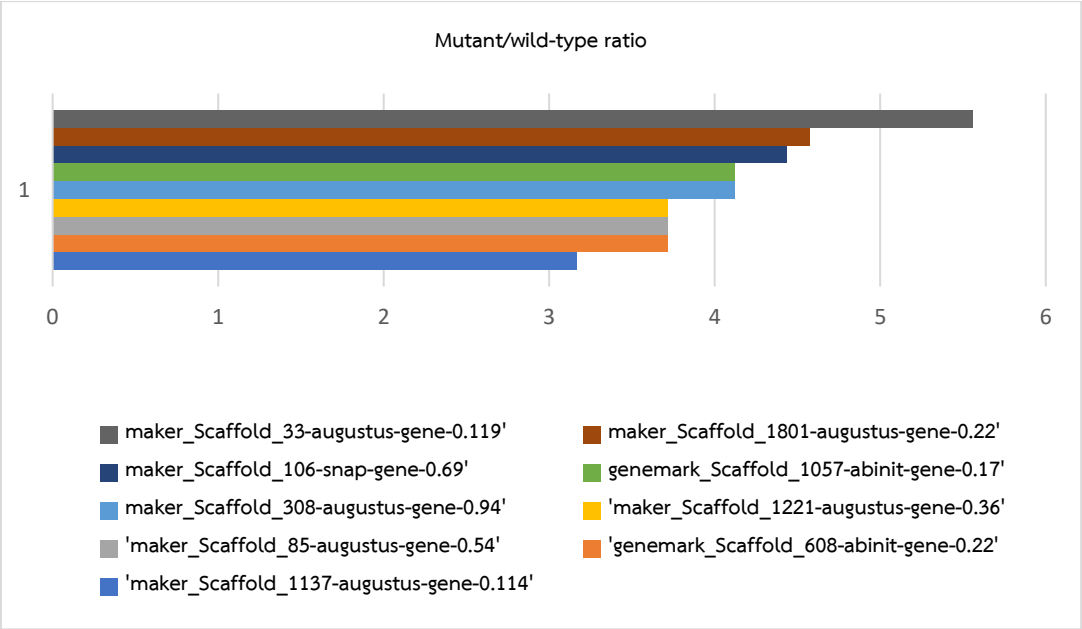

Figure 5 Mutant/wild-type ratios for top nine potential knocked-out genes as their mutants yield higher than three-fold compared to the wild-type (mixotrophy).

Table 10 Mutant/wildtype ratio under mixotrophic condition

Gene List	Mutant/wildtype ratio
'maker_Scaffold_33-augustus-gene-0.119'	5.557828452
'maker_Scaffold_1801-augustus-gene-0.22'	4.571113008
'maker_Scaffold_106-snap-gene-0.69'	4.437901059
'genemark_Scaffold_1057-abinit-gene-0.17'	4.119651827
'maker_Scaffold_308-augustus-gene-0.94'	4.119647483
'maker_Scaffold_85-augustus-gene-0.54'	3.717476863
'maker_Scaffold_1221-augustus-gene-0.36'	3.717476863
'genemark_Scaffold_608-abinit-gene-0.22'	3.71741952
'maker_Scaffold_1137-augustus-gene-0.114'	3.169098674
'genemark_Scaffold_335-abinit-gene-0.8'	1.140335345

4.2.4 β-oxidation pathway

The process of beta-oxidation involves breaking down fatty acids into acetyl-CoA molecules, which can then be utilized for energy production via the citric acid cycle. Fatty acid degradation, which involves the process of beta-oxidation, this conserved metabolic process known to occur in peroxisomes of land plants and yeast, as well as in both peroxisomes and mitochondria in mammalian cells. In 2017, Kong, F., et al. demonstrated that inhibiting key enzymes involved in the β-oxidation pathway of fatty acid metabolism can increase the oil content in *Chlamydomonas reinhardtii*.

In addition, Kato et al. in 2013 showed that inhibition of lipid degradation in *Chlamydomonas* increase in TAG content due to delayed FA β-oxidation. Our study showed many predicted genes increased in TAG after single-gene deletion of genes involved in β-oxidation such as genemark_Scaffold_1016-abinit-gene-0.16, dgenemark_Scaffold_1220-abinit-gene-0.13(heterotrophy) and maker_Scaffold_33-augustus-gene-0.119(mixotrophy). However, it has been shown that the degradation of fatty acids in microalgae depends on their phylogenetic evolution, and the understanding of β-oxidation in microalgae is limited due to the limited literature [68, 74]. Figure 6 and Figure 7 show a schematic representation of the life cycle of lipid droplets in *Chlamydomonas* along with the lipid degradation steps before entering the β-oxidation pathway. This resulted in a decrease in TAG [75].

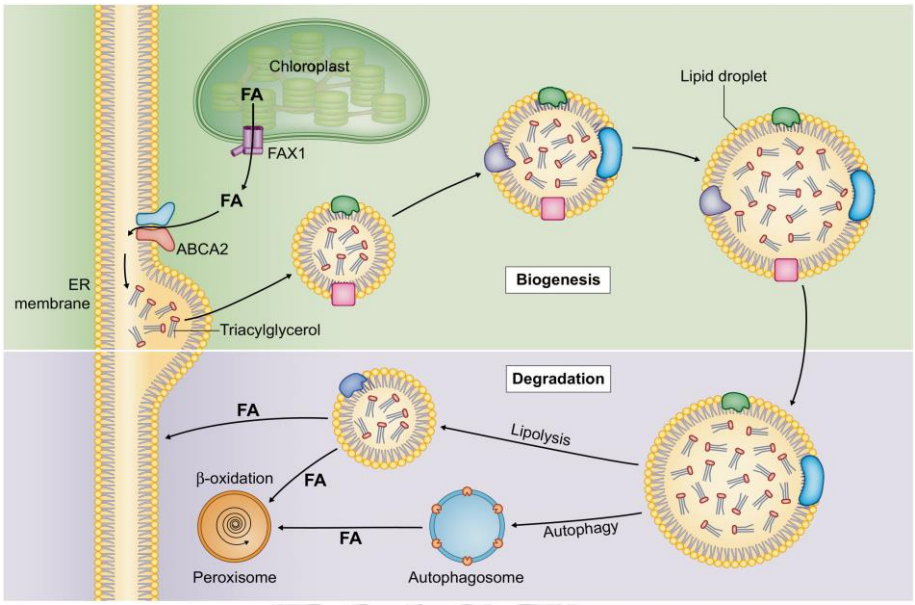

Figure 6 The diagram illustrates the life cycle of a lipid droplet in Chlamydomonas [75]. *The diagram illustrates the life cycle of a lipid droplet in* Chlamydomonas. *Fatty acids and diacylglycerol (DAG) precursors, provided by the chloroplast, are used in the endoplasmic reticulum (ER) for the assembly of triacylglycerols (TAGs), resulting in the formation of lipid droplets (LDs). The transport of fatty acids from the chloroplast to the ER is facilitated by fatty acid export1 (fax1) [76], while their import into the ER is mediated by atp-binding cassette transporter (ABCA2)* [77], *which functions similarly to their counterparts in the land plant* Arabidopsis thaliana. *LDs can undergo degradation through two parallel pathways: lipolysis or autophagy. Degraded LDs release TAGs, which are then broken down by lipases, resulting in the production of free fatty acids. These fatty acids can be utilized for membrane reassembly or serve as carbon or energy precursors through β-oxidation in the peroxisome. It's important to note that, for simplicity, the processes of LD biogenesis and degradation are depicted side by side in the diagram, although they are typically temporally separated depending on developmental or environmental signals. FA refers to fatty acid.*

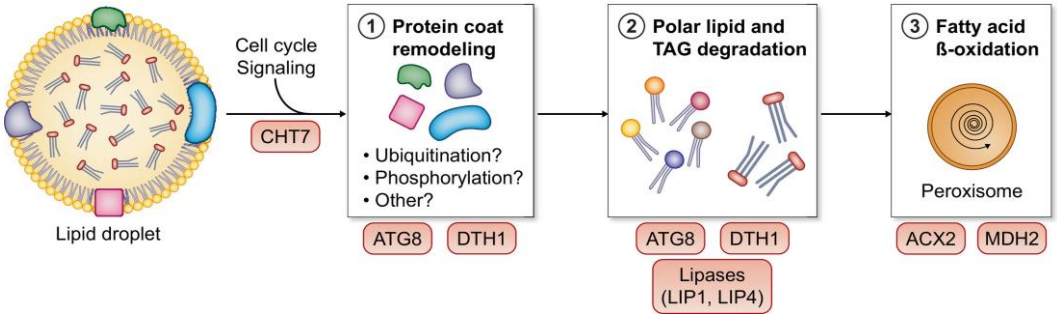

Figure 7 Theoretical steps of lipid droplet degradation. [78]

The process of lipid droplet degradation involves several steps, starting from initiation and proceeding through protein coat remodeling, degradation of polar lipids and triacylglycerols (TAGs), and ultimately, the β-oxidation of fatty acids. The major proteins associated with each step are indicated. These include acx2 (acyl-coa oxidase2), atg8 (autophagy-related protein8), cht7 (compromised in tag hydrolysis7), dth1 (delayed in tag hydrolysis1), lip1/lip4 (lipase1/lipase4), and mdh2 (malate dehydrogenase2).

CHAPTER 5 CONCLUSION AND RECOMMENDATIONS

In this study, LMOMA is shown to be a more appropriate tool for gene perturbation prediction than FBA. LMOMA suggested that highest TAG production after single-gene deletion prediction was eight times in heterotrophy ('genemark_Scaffold_1016-abinit-gene-0.16'; cofactor recycling), seven times in photoautotrophy ('maker_Scaffold_332-augustus-gene-0.46'; galactose metabolism), and five times in mixotrophy ('maker_Scaffold_33-augustus-gene-0.119'; TCA cycle; carbon fixation; CO2 fixation). Also, the results suggested that manipulating fatty acid β-oxidation in *C. vulgaris* might be a valuable strategy to develop the high TAG algal strains, which can be utilized for applications including biofuel, nutrition, and eco-friendly applications.

Limitation of this study

Although this study found that fatty acid β-oxidation in *C. vulgaris* was an interesting pathway for genetic manipulation, the knowledge of this pathway in *C. vulgaris* is limited, and it does not resemble that of mammals and land plants. In addition, the model used in this study was not solely constructed from *C. vulgaris*, but was from a combination of a few algal taxa *C. variabilis*, *C. subellipsoidea*, and *C. reinhardtii*. Therefore, it might not truly reflect that reactions present in *C. vulgaris*.

APPENDICES

APPENDIX A

APPENDIX A 55 potential gene after single gene deletion using LMOMA, Heterotrophy

Genes	Knocked-out genes flux value	Mutant/wildtype ratio	Reaction name in iCZ843	Reaction description	Formula	Subsystem	EC Number	KEGG ID	References
genemark_Scaffold_37-abinit-gene-0.8'	0.001824679	1.000000001	AGAKI	alpha-D-Glucose aldose-ketose-isomerase	glc-A[h] <=> fru-B[h]	Fructose and mannose metabolism	5.3.1.5	'R00878'	
			DRBK	ribokinase (deoxyribose)	atp[c] + drib[c] -> 2dr5p[c] + adp[c] + h[c]	Pentose phosphate pathway	2.7.1.15	'R02750'	
			RBK	Ribokinase (ribose)	atp[c] + rib-D[c] -> adp[c] + h[c] + r5p[c]	Pentose phosphate pathway	2.7.1.15	'R01051'	
snap_masked_Scaffold_291-abinit-gene-0.6'	0.001824679	1.000000001	NA1ATPasem	Na+-translocating F-type ATPase, mitochondrial	atp[m] + h2o[m] + na1[m] -> adp[m] + h[m] + na1[c] + pi[m]	Transport, mitochondria	3.A.2.1.2		[79]
			ATPSm	F0F1-ATP synthase Complex V	adp[m] + 3 h[c] + pi[m] -> atp[m] + 2 h[m] + h2o[m]	Oxidative phosphorylation	3.6.3.14	'R00086'	[80]
			ATPSh	ATP synthase	adp[h] + 4 h[u] + pi[h] -> atp[h] + 3 h[h] + h2o[u]	Photosynthesis	3.6.3.14	'R00086'	[80]
augustus_masked_Scaffold_1130-abinit-gene-0.2'	0.001824679	1.000000001	ATPSm	F0F1-ATP synthase Complex V	adp[m] + 3 h[c] + pi[m] -> atp[m] + 2 h[m] + h2o[m]	Oxidative phosphorylation	3.6.3.14	'R00086'	[80]
			ATPSh	ATP synthase	adp[h] + 4 h[u] + pi[h]	Photosynthes	3.6.3.14	'R0008	[80]

Genes	Knocked-out genes flux value	Mutant/wildtype ratio	Reaction name in iCZ843	Reaction description	Formula	Subsystem	EC Number	KEGG ID	References
					-> atp[h] + 3 h[h] + h2o[u]	is		6'	
genemark_Scaffold_1087-abinit-gene-0.4'	0.001824679	1.000000001	ATPSm	F0F1-ATP synthase Complex V	adp[m] + 3 h[c] + pi[m] -> atp[m] + 2 h[m] + h2o[m]	Oxidative phosphorylation	3.6.3.14	'R00086'	[80]
			ATPSh	ATP synthase	adp[h] + 4 h[u] + pi[h] -> atp[h] + 3 h[h] + h2o[u]	Photosynthesis	3.6.3.14	'R00086'	[80]
maker_Scaffold_1038-augustus-gene-0.43'	0.001824679	1.000000001	ATPSm	F0F1-ATP synthase Complex V	adp[m] + 3 h[c] + pi[m] -> atp[m] + 2 h[m] + h2o[m]	Oxidative phosphorylation	3.6.3.14	'R00086'	[80]
			ATPSh	ATP synthase	adp[h] + 4 h[u] + pi[h] -> atp[h] + 3 h[h] + h2o[u]	Photosynthesis	3.6.3.14	'R00086'	[80]
maker_Scaffold_156-augustus-gene-0.44'	0.001824679	1.000000001	ATPSm	F0F1-ATP synthase Complex V	adp[m] + 3 h[c] + pi[m] -> atp[m] + 2 h[m] + h2o[m]	Oxidative phosphorylation	3.6.3.14	'R00086'	[80]
			ATPSh	ATP synthase	adp[h] + 4 h[u] + pi[h] -> atp[h] + 3 h[h] + h2o[u]	Photosynthesis	3.6.3.14	'R00086'	[80]
maker_Scaffold_685-augustus-gene-0.113'	0.001824679	1.000000001	ATPSm	F0F1-ATP synthase Complex V	adp[m] + 3 h[c] + pi[m] -> atp[m] + 2 h[m] + h2o[m]	Oxidative phosphorylation	3.6.3.14	'R00086'	[80]
			ATPSh	ATP synthase	adp[h] + 4 h[u] + pi[h] -> atp[h] + 3 h[h] + h2o[u]	Photosynthesis	3.6.3.14	'R00086'	[80]

Genes	Knocked-out genes flux value	Mutant/wildtype ratio	Reaction name in iCZ843	Reaction description	Formula	Subsystem	EC Number	KEGG ID	References
maker_Scaffold_2053-augustus-gene-0.13'	0.001824679	1.000000001	ATCY	ATP:cytidine 5'-phosphotransferase	atp[c] + cytd[c] -> adp[c] + cmp[c] + h[c]	Pyrimidine metabolism	2.7.1.48	'R00513'	
			AUPT	ATP:uridine 5'-phosphotransferase	atp[c] + uri[c] -> adp[c] + h[c] + ump[c]	Pyrimidine metabolism	2.7.1.48	'R00964'	
			DATCY	dATP:cytidine 5'-phosphotransferase	cytd[c] + datp[c] -> cmp[c] + dadp[c] + h[c]	Pyrimidine metabolism	2.7.1.48	'R01548'	
			DATUP	dATP:uridine 5'-phosphotransferase	datp[c] + uri[c] -> dadp[c] + h[c] + ump[c]	Pyrimidine metabolism	2.7.1.48	'R01549'	
			DCTCP	dCTP:cytidine 5'-phosphotransferase	cytd[c] + dctp[c] -> cmp[c] + dcdp[c] + h[c]	Pyrimidine metabolism	2.7.1.48	'R02371'	
			DCTUP	dCTP:uridine 5'-phosphotransferase	dctp[c] + uri[c] -> dcdp[c] + h[c] + ump[c]	Pyrimidine metabolism	2.7.1.48	'R02327'	
			DGTCY	dGTP:cytidine 5'-phosphotransferase	cytd[c] + dgtp[c] -> cmp[c] + dgdp[c] + h[c]	Pyrimidine metabolism	2.7.1.48	'R02091'	
			DGTUP	dGTP:uridine 5'-phosphotransferase	dgtp[c] + uri[c] -> dgdp[c] + h[c] + ump[c]	Pyrimidine metabolism	2.7.1.48	'R01880'	
			DTTGY	dTTP:cytidine 5'-phosphotransferase	cytd[c] + dttp[c] -> cmp[c] + dtdp[c] + h[c]	Pyrimidine metabolism	2.7.1.48	'R02096'	

Genes	Knocked-out genes flux value	Mutant/wildtype ratio	Reaction name in iCZ843	Reaction description	Formula	Subsystem	EC Number	KEGG ID	References
			DTTUP	dTTP:uridine 5'-phosphotransferase	dttp[c] + uri[c] -> dtdp[c] + h[c] + ump[c]	Pyrimidine metabolism	2.7.1.48	'R02097'	
			DUTCP	dUTP:cytidine 5'-phosphotransferase	cytd[c] + dutp[c] -> cmp[c] + dudp[c] + h[c]	Pyrimidine metabolism	2.7.1.48	'R02372'	
			DUTUP	dUTP:uridine 5'-phosphotransferase	dutp[c] + uri[c] -> dudp[c] + h[c] + ump[c]	Pyrimidine metabolism	2.7.1.48	'R02332'	
			GTCY	GTP:cytidine 5'-phosphotransferase	cytd[c] + gtp[c] -> cmp[c] + gdp[c] + h[c]	Pyrimidine metabolism	2.7.1.48	'R00517'	
			GTUP	GTP:uridine 5'-phosphotransferase	gtp[c] + uri[c] -> gdp[c] + h[c] + ump[c]	Pyrimidine metabolism	2.7.1.48	'R00968'	
			ITCY	ITP:cytidine 5'-phosphotransferase	cytd[c] + itp[c] -> cmp[c] + h[c] + idp[c]	Pyrimidine metabolism	2.7.1.48	'R00962'	
			ITUP	ITP:uridine 5'-phosphotransferase	itp[c] + uri[c] -> h[c] + idp[c] + ump[c]	Pyrimidine metabolism	2.7.1.48	'R00970'	
			UTCY	UTP:cytidine 5'-phosphotransferase	cytd[c] + utp[c] -> cmp[c] + h[c] + udp[c]	Pyrimidine metabolism	2.7.1.48	'R00516'	
			UTUP	UTP:uridine 5'-phosphotransferase	uri[c] + utp[c] -> h[c] + udp[c] + ump[c]	Pyrimidine metabolism	2.7.1.48	'R00967'	
genemark_Scaffold_366-abinit-gene-0.45'	0.001824679	1.000000006	SHSL2h	O-succinyl-L-homoserine succinate-lyase (adding hydrogen sulfide)	h2s[h] + suchms[h] -> hcys-L[h] + succ[h]	Cysteine and methionine metabolism	2.5.1.48;2.5.1.-	'R01288'	

Genes	Knocked-out genes flux value	Mutant/wildtype ratio	Reaction name in iCZ843	Reaction description	Formula	Subsystem	EC Number	KEGG ID	References
			DEDOLDPS	dehydrodolichol diphosphate synthase	7 ipdp[c] + ttc-ggdp[c] -> dedoldp[c] + 7 ppi[c]	Terpenoid backbone biosynthesis	2.5.1.-	'R05556'	
maker_Scaffold_504-augustus-gene-0.24'	0.001824679	1.000000006	DEDOLDPSAT	dehydrodolichol diphosphate saturase	dedoldp[c] + h[c] + nadh[c] -> doldp[c] + nad[c]	Terpenoid backbone biosynthesis		'R08375'	
			TTCGGDPS	trans,trans,cis-geranylgeranyl diphosphate synthase	frdp[c] + ipdp[c] -> ppi[c] + ttc-ggdp[c]	Terpenoid backbone biosynthesis	2.5.1.31	'R05555'	
maker_Scaffold_1358-augustus-gene-0.13'	0.001824679	1.000000007	MDH(nadp)hi	malate dehydrogenase (NADP+)	h[h] + nadph[h] + oaa[h] -> mal-L[h] + nadp[h]	Carbon fixation	1.1.1.82	'R00343'	[81, 82]
			mdh(nadp)Cv	malate dehydrogenase, chloroplast (NADP)	mal-L[h] + nadp[h] -> h[h] + nadph[h] + oaa[h]	TCA cycle	3.6.1.18	'R00343'	[81, 82]
maker_Scaffold_374-augustus-gene-0.178'	0.001824679	1.000000012	NAPT	nicotinamide phosphoribosyltransferase	nmn[c] + ppi[c] <=> ncam[c] + prpp[c]	Nicotinate and nicotinamide metabolism	2.4.2.12	'R01271'	
maker_Scaffold_1137-snap-gene-0.128'	0.001824679	1.00000002	PGMTh	phosphoglucomutase, chloroplast	g1p[h] <=> g6p-A[h]	Glycolysis / Gluconeogenesis	5.4.2.2	'R00959'	[83]
			PPMh	Phosphopentosemutase	r1p[h] <=> r5p[h]	Pentose phosphate pathway	5.4.2.2	'R01057'	[83]
maker_Scaffold_916-snap-gene-0.4'	0.001824679	1.000000059	AKGCITtm	Dicarboxylate/tricarboxylate carrier (akg:cit), mitochondrial	akg[c] + cit[m] <=> akg[m] + cit[c]	Transport, mitochondria	2.A.29.2.6		[84]

Genes	Knocked-out genes flux value	Mutant/wildtype ratio	Reaction name in iCZ843	Reaction description	Formula	Subsystem	EC Number	KEGG ID	References
			AKGICITtm	Dicarboxylate/tricarboxylate carrier (akg:icit), mitochondrial	akg[c] + icit[m] <=> akg[m] + icit[c]	Transport, mitochondria	2.A.29.2.6		[84]
			CITICITtm	Dicarboxylate/tricarboxylate carrier (cit:icit), mitochondrial	cit[c] + icit[m] <=> cit[m] + icit[c]	Transport, mitochondria	2.A.29.2.6		[84]
			MALAKGtm	Dicarboxylate/tricarboxylate carrier (mal:akg), mitochondrial	akg[m] + mal-L[c] <=> akg[c] + mal-L[m]	Transport, mitochondria	2.A.29.2.6		[84]
			MALCITtm	Dicarboxylate/tricarboxylate carrier (mal:cit), mitochondrial	cit[m] + mal-L[c] <=> cit[c] + mal-L[m]	Transport, mitochondria	2.A.29.2.6		[84]
			MALICITtm	Dicarboxylate/tricarboxylate carrier (mal:icit), mitochondrial	icit[m] + mal-L[c] <=> icit[c] + mal-L[m]	Transport, mitochondria	2.A.29.2.6		[84]
			MALOAAtm	Dicarboxylate/tricarboxylate carrier (mal:oaa), mitochondrial	mal-L[m] + oaa[c] <=> mal-L[c] + oaa[m]	Transport, mitochondria	2.A.29.2.6		[84]
			OAAAKGtm	Dicarboxylate/tricarboxylate carrier (oaa:akg), mitochondrial	akg[m] + oaa[c] <=> akg[c] + oaa[m]	Transport, mitochondria	2.A.29.2.6		[84]
			OAACITtm	Dicarboxylate/tricarboxylate carrier (oaa:cit), mitochondrial	cit[m] + oaa[c] <=> cit[c] + oaa[m]	Transport, mitochondria	2.A.29.2.6		[84]
			OAAICITtm	Dicarboxylate/tricarboxylate carrier (oaa:icit),	icit[m] + oaa[c] <=> icit[c] + oaa[m]	Transport, mitochondria	2.A.29.2.6		[84]

Genes	Knocked-out genes flux value	Mutant/wildtype ratio	Reaction name in iCZ843	Reaction description	Formula	Subsystem	EC Number	KEGG ID	References
				mitochondrial					
snap_masked_Scaffold_556-abinit-gene-0.35'	0.001824679	1.000000066	M5TAP	S-methyl-5'-thioadenosine phosphorylase	5mta[c] + pi[c] -> 5mdr1p[c] + ade[c]	Methionine metabolism	2.4.2.28	'R01402'	
			upCv	uridine phosphorylase	pi[c] + uri[c] <=> r1p[c] + ura[c]	Pyrimidine metabolism	2.4.2.3	'R01876'	
maker_Scaffold_208-augustus-gene-0.149'	0.001824679	1.000000067	NA1Hth	Na+/H+ antiporter, chloroplast	h[h] + na1[c] <=> h[c] + na1[h]	Transport, chloroplast	2.A.62.1.1;2.A.36.2.1		
			NA1Htm	Na+/H+ antiporter, mitochondrial	h[m] + na1[c] <=> h[c] + na1[m]	Transport, mitochondria	2.A.62.1.1;2.A.36.2.1		
augustus_masked_Scaffold_755-abinit-gene-0.7'	0.001824679	1.00000027	GLYPT	ATP:(R)-glycerate 3-phosphotransferase	atp[h] + glyc-R[h] -> 3pg[h] + adp[h] + h[h]	Glycine, serine and threonine metabolism	2.7.1.31	'R01514'	
			GLYCK	Glycerate kinase	atp[c] + glyc-R[c] -> 3pg[c] + adp[c] + h[c]	Glyoxylate metabolism	2.7.1.31	'R01514'	
maker_Scaffold_772-augustus-gene-0.100'	0.00182559	1.000499218	AM6PT	ATP:D-mannose 6-phosphotransferase	atp[c] + man[c] -> adp[c] + h[c] + man6p[c]	Fructose and mannose metabolism	2.7.1.1;2.7.1.2	'R01326'	[85, 86]
			AM6PTh	ATP:D-mannose 6-phosphotransferase, chloroplast	atp[h] + man[h] -> adp[h] + h[h] + man6p[h]	Fructose and mannose metabolism	2.7.1.1;2.7.1.2	'R01326'	[85, 86]
			GLUKA	Glucokinase/hexokinase (glc-A)	atp[c] + glc-A[c] -> adp[c] + g6p-A[c] +	Glycolysis / Gluconeogen	2.7.1.1;2.7.1.2	'R01786'	[85, 86]

Genes	Knocked-out genes flux value	Mutant/wildtype ratio	Reaction name in iCZ843	Reaction description	Formula	Subsystem	EC Number	KEGG ID	References
					h[c]	esis			
			GLUKAh	Glucokinase/hexokinase, chloroplast (glc-A)	atp[h] + glc-A[h] -> adp[h] + g6p-A[h] + h[h]	Glycolysis / Gluconeogenesis	2.7.1.1;2.7.1.2	'R01786'	[85, 86]
			GLUKB	Glucokinase/hexokinase (glc-B)	atp[c] + glc-B[c] -> adp[c] + g6p-B[c] + h[c]	Glycolysis / Gluconeogenesis	2.7.1.1;2.7.1.2	'R01600'	[85, 86]
			GLUKBh	Glucokinase/hexokinase, chloroplast (glc-B)	atp[h] + glc-B[h] -> adp[h] + g6p-B[h] + h[h]	Glycolysis / Gluconeogenesis	2.7.1.1;2.7.1.2	'R01600'	[85, 86]
			ABFPT	ATP:D-fructose 6-phosphotransferase	atp[c] + fru-B[c] -> adp[c] + f6p-B[c] + h[c]	Starch and sucrose metabolism	2.7.1.1;2.7.1.2;2.7.1.4	'R03920'	[85, 86]
			ABFPTh	ATP:D-fructose 6-phosphotransferase, chloroplast	atp[h] + fru-B[h] -> adp[h] + f6p-B[h] + h[h]	Starch and sucrose metabolism	2.7.1.1;2.7.1.2;2.7.1.4	'R03920'	[85, 86]
maker_Scaffold_141-augustus-gene-0.146'	0.001827556	1.001576807	NA1th	Voltage-sensitive Na+ channel, chloroplast	na1[c] <=> na1[h]	Transport, chloroplast	1.A.1.10.1		
			NA1tm	Voltage-sensitive Na+ channel, mitochondrial	na1[c] <=> na1[m]	Transport, mitochondria	1.A.1.10.1		
maker_Scaffold_93-augustus-gene-0.49'	0.003317807	1.818296335	OCT	ornithine carbamoyltransferase	cbp[c] + orn[c] <=> citr-L[c] + pi[c]	Arginine and proline metabolism; Urea cycle and metabolism	2.1.3.3	'R01398'	

Genes	Knocked-out genes flux value	Mutant/wildtype ratio	Reaction name in iCZ843	Reaction description	Formula	Subsystem	EC Number	KEGG ID	References
						of amino groups			
			OCTh	ornithine carbamoyltransferase, chloroplast	cbp[h] + orn[h] <=> citr-L[h] + pi[h]	Arginine and proline metabolism; Urea cycle and metabolism of amino groups	2.1.3.3	'R01398'	
			OCTm	ornithine carbamoyltransferase, mitochondrial	cbp[m] + orn[m] <=> citr-L[m] + pi[m]	Arginine and proline metabolism; Urea cycle and metabolism of amino groups	2.1.3.3	'R01398'	
			cpoCv	carbamoyl-phosphate:N2-acetyl-L-ornithine carbamoyltransferase	acorn[c] + cbp[c] <=> h[c] + pi[c] + accitl[c]	Arginine and proline metabolism	2.1.3.9	'R07245'	
maker_Scaffold_21-augustus-gene-0.94'	0.005187107	2.8427507	AIAL	1-(5'-Phosphoribosyl)-5-amino-4-(N-succinocarboxamide)-imidazole AMP-lyase	25aics[c] <=> aicar[c] + fum[c]	Purine metabolism	4.3.2.2	'R04559'	

Genes	Knocked-out genes flux value	Mutant/wildtype ratio	Reaction name in iCZ843	Reaction description	Formula	Subsystem	EC Number	KEGG ID	References
			FUMm	fumarate hydratase	fum[m] + h2o[m] <=> mal-L[m]	TCA cycle;CO2 fixation	4.2.1.2	'R01082'	
maker_Scaffold_645-augustus-gene-0.44'	0.005189518	2.844072255	ACCOAth	Acetyl-CoA:CoA antiporter, chloroplast	accoa[c] + coa[h] <=> accoa[h] + coa[c]	Transport, chloroplast	2.A.1.25.1		[79]
			ACCOAtx	Acetyl-CoA:CoA antiporter, glyoxysomal	accoa[c] + coa[x] <=> accoa[x] + coa[c]	Transport, glyoxysome	2.A.1.25.1		[79]
			ACCOAtm	Acetyl-CoA:CoA antiporter, mitochondrial	accoa[c] + coa[m] <=> accoa[m] + coa[c]	Transport, mitochondria	2.A.1.25.1		
	0.005335842	2.924263731	MGDGS1819Z160	monogalactosyldiacylglycerol synthase (18:1(9Z)/16:0)	12dgr1819Z160[h] + udpgal[h] -> h[h] + mgdg1819Z160[h] + udp[h]	Glycerolipid metabolism	2.4.1.46	'R02691'	[87, 88]
			MGDGS1819Z1619Z	monogalactosyldiacylglycerol synthase (18:1(9Z)/16:1(9Z))	12dgr1819Z1619Z[h] + udpgal[h] -> h[h] + mgdg1819Z1619Z[h] + udp[h]	Glycerolipid metabolism	2.4.1.46	'R02691'	[87, 88]
maker_Scaffold_329-augustus-gene-0.180'	0.005741777	3.146733379	FA100ACPHi	fatty-acyl-ACP hydrolase (n-C10:0)	dcaACP[h] + h2o[h] -> ACP[h] + dca[h] + h[h]	Fatty acid biosynthesis	3.1.2.14	'R08158'	[87, 88]
			FA120ACPHi	fatty-acyl-ACP hydrolase (n-C12:0)	ddcaACP[h] + h2o[h] -> ACP[h] + ddca[h] + h[h]	Fatty acid biosynthesis	3.1.2.14	'R04014'	[87, 88]
			FA140ACPHi	fatty-acyl-ACP hydrolase (n-C14:0)	h2o[h] + myrsACP[h] -> ACP[h] + h[h] +	Fatty acid biosynthesis	3.1.2.14	'R08159'	[87, 88]

Genes	Knocked-out genes flux value	Mutant/wildtype ratio	Reaction name in iCZ843	Reaction description	Formula	Subsystem	EC Number	KEGG ID	References
					ttdca[h]				
			FA160ACPHi	fatty-acyl-ACP hydrolase (n-C16:0)	h2o[h] + palmACP[h] -> ACP[h] + h[h] + hdca[h]	Fatty acid biosynthesis	3.1.2.14;2.3.1.-	'R01706'	[87, 88]
			FA161ACPHi	fatty-acyl-ACP hydrolase ((9Z)-n-C16:1)	h2o[h] + hdeACP[h] -> ACP[h] + h[h] + hdcea[h]	Fatty acid biosynthesis	3.1.2.14	'R08162'	[87, 88]
			FA180ACPHi	fatty-acyl-ACP hydrolase (n-C18:0)	h2o[h] + ocdcaACP[h] -> ACP[h] + h[h] + ocdca[h]	Fatty acid biosynthesis	3.1.2.14;2.3.1.-	'R08163'	[87, 88]
			FA1819ZACPH	oleoyl-[acyl-carrier-protein] hydrolase ((9Z)-n-C18:1)	h2o[h] + octe9ACP[h] -> ACP[h] + h[h] + ocdce9a[h]	Fatty acid biosynthesis	3.1.2.14	'R02814'	[87, 88]
			FA181ACPHi	oleoyl-[acyl-carrier-protein] hydrolase ((11Z)-n-C18:1)	h2o[h] + octeACP[h] -> ACP[h] + h[h] + ocdcea[h]	Fatty acid biosynthesis	3.1.2.14		[87, 88]
			FA80ACPHi	fatty-acyl-ACP hydrolase (n-C8:0)	h2o[h] + ocACP[h] -> ACP[h] + h[h] + octa[h]	Fatty acid biosynthesis	3.1.2.14	'R08157'	[87, 88]
augustus_masked_Scaffold_79-abinit-gene-0.2'	0.00606069	3.32151061	ACPT16018111Z	betaine lipid synthase (3-amino-3-carboxypropyltransferase) (16:0/18:1(11Z))	12dgr16018111Z[c] + amet[c] -> 5mta[c] + dghs16018111Z[c] + h[c]	Glycerolipid metabolism			
			ACPT1601819Z	betaine lipid synthase (3-amino-3-carboxypropyltransferas	12dgr1601819Z[c] + amet[c] -> 5mta[c] + dghs1601819Z[c] +	Glycerolipid metabolism			

Genes	Knocked-out genes flux value	Mutant/wildtype ratio	Reaction name in iCZ843	Reaction description	Formula	Subsystem	EC Number	KEGG ID	References
				e) (16:0/18:1(9Z))	h[c]				
			ACPT18111Z18111Z	betaine lipid synthase (3-amino-3-carboxypropyltransferase) (18:1(11Z)/18:1(11Z))	12dgr18111Z18111Z[c] + amet[c] -> 5mta[c] + dghs18111Z18111Z[c] + h[c]	Glycerolipid metabolism			
			ACPT18111Z1819Z	betaine lipid synthase (3-amino-3-carboxypropyltransferase) (18:1(11Z)/18:1(9Z))	12dgr18111Z1819Z[c] + amet[c] -> 5mta[c] + dghs18111Z1819Z[c] + h[c]	Glycerolipid metabolism			
			ACPT1819Z18111Z	betaine lipid synthase (3-amino-3-carboxypropyltransferase) (18:1(9Z)/18:1(11Z))	12dgr1819Z18111Z[c] + amet[c] -> 5mta[c] + dghs1819Z18111Z[c] + h[c]	Glycerolipid metabolism			
			ACPT1819Z1819Z	betaine lipid synthase (3-amino-3-carboxypropyltransferase) (18:1(9Z)/18:1(9Z))	12dgr1819Z1819Z[c] + amet[c] -> 5mta[c] + dghs1819Z1819Z[c] + h[c]	Glycerolipid metabolism			
			TM16018111Z	betaine lipid synthase (trimethylase) (16:0/18:1(11Z))	3 amet[c] + dghs16018111Z[c] -> 3 ahcys[c] + dgts16018111Z[c] + 3 h[c]	Glycerolipid metabolism			
			TM1601819Z	betaine lipid synthase	3 amet[c] +	Glycerolipid			

Genes	Knocked-out genes flux value	Mutant/wildtype ratio	Reaction name in iCZ843	Reaction description	Formula	Subsystem	EC Number	KEGG ID	References
				(trimethylase) (16:0/18:1(9Z))	dghs1601819Z[c] -> 3 ahcys[c] + dgts1601819Z[c] + 3 h[c]	metabolism			
			TM18111Z18111Z	betaine lipid synthase (trimethylase) (18:1(11Z)/18:1(11Z))	3 amet[c] + dghs18111Z18111Z[c] -> 3 ahcys[c] + dgts18111Z18111Z[c] + 3 h[c]	Glycerolipid metabolism			
			TM18111Z1819Z	betaine lipid synthase (trimethylase) (18:1(11Z)/18:1(9Z))	3 amet[c] + dghs18111Z1819Z[c] -> 3 ahcys[c] + dgts18111Z1819Z[c] + 3 h[c]	Glycerolipid metabolism			
			TM1819Z18111Z	betaine lipid synthase (trimethylase) (18:1(9Z)/18:1(11Z))	3 amet[c] + dghs1819Z18111Z[c] -> 3 ahcys[c] + dgts1819Z18111Z[c] + 3 h[c]	Glycerolipid metabolism			
			TM1819Z1819Z	betaine lipid synthase (trimethylase) (18:1(9Z)/18:1(9Z))	3 amet[c] + dghs1819Z1819Z[c] -> 3 ahcys[c] + dgts1819Z1819Z[c] + 3 h[c]	Glycerolipid metabolism			
maker_Scaffold_2123-augustus-gene-0.11'	0.006117657	3.352731226	G1SAT	glutamate-1-semialdehyde	glu1sa[h] <=> 5aop[h] + h[h]	Porphyrin and	5.4.3.8	'R02272'	[89]

Genes	Knocked-out genes flux value	Mutant/wildtype ratio	Reaction name in iCZ843	Reaction description	Formula	Subsystem	EC Number	KEGG ID	References
				aminotransferase		chlorophyll metabolism			
maker_Scaffold_137-augustus-gene-0.65'	0.006117657	3.352731226	GLUTRR	glutamyl-tRNA reductase	glutrna[h] + h[h] + nadph[h] -> glu1sa[h] + nadp[h] + trnaglu[h]	Porphyrin and chlorophyll metabolism	1.2.1.70	'R04109'	[89]
maker_Scaffold_1615-augustus-gene-0.55'	0.006117657	3.352731226	PPBNGD	porphobilinogen deaminase	h2o[h] + 4 ppbng[h] <=> hmbil[h] + 4 nh4[h]	Porphyrin and chlorophyll metabolism	2.5.1.61	'R00084'	
maker_Scaffold_119-augustus-gene-0.94'	0.006117657	3.352731226	PPBNGS	porphobilinogen synthase	2 5aop[h] + h[h] -> 2 h2o[h] + ppbng[h]	Porphyrin and chlorophyll metabolism	4.2.1.24	'R00036'	[90]
maker_Scaffold_758-augustus-gene-0.38'	0.006117657	3.352731226	UPP3S	uroporphyrinogen III synthase	hmbil[h] -> h2o[h] + uppg3[h]	Porphyrin and chlorophyll metabolism	4.2.1.75	'R03165'	
augustus_masked_Scaffold_835-abinit-gene-0.5'	0.006188586	3.391603054	CPS	carbamoyl-phosphate synthase (ammonia)	2 atp[h] + co2[h] + h2o[h] + nh4[h] -> 2 adp[h] + cbp[h] + 3 h[h] + pi[h]	Nitrogen metabolism	6.3.4.16	'R00149'	
			HCGAL	hydrogen-carbonate:L-glutamine amido-ligase	2 atp[c] + gln-L[c] + h2o[c] + hco3[c] -> 2 adp[c] + cbp[c] + glu-L[c] + 2 h[c] + pi[c]	Pyrimidine metabolism	6.3.5.5	'R00575'	

Genes	Knocked-out genes flux value	Mutant/wildtype ratio	Reaction name in iCZ843	Reaction description	Formula	Subsystem	EC Number	KEGG ID	References
			HCGALm	hydrogen-carbonate:L-glutamine amido-ligase, mitochondria	2 atp[m] + gln-L[m] + h2o[m] + hco3[m] -> 2 adp[m] + cbp[m] + glu-L[m] + 2 h[m] + pi[m]	Pyrimidine metabolism	6.3.5.5	'R00575'	
maker_Scaffold_556-augustus-gene-0.46'	0.006188586	3.391603054	HCGAL	hydrogen-carbonate:L-glutamine amido-ligase	2 atp[c] + gln-L[c] + h2o[c] + hco3[c] -> 2 adp[c] + cbp[c] + glu-L[c] + 2 h[c] + pi[c]	Pyrimidine metabolism	6.3.5.5	'R00575'	
			HCGALm	hydrogen-carbonate:L-glutamine amido-ligase, mitochondria	2 atp[m] + gln-L[m] + h2o[m] + hco3[m] -> 2 adp[m] + cbp[m] + glu-L[m] + 2 h[m] + pi[m]	Pyrimidine metabolism	6.3.5.5	'R00575'	
maker_Scaffold_899-augustus-gene-0.126'	0.006188586	3.391603054	H2Oth	H2O transport, chloroplast	h2o[c] <=> h2o[h]	Transport, chloroplast	1.A.8.11.1		[79]
			H2Ot	H2O transport, extracellular	h2o[e] <=> h2o[c]	Transport, extracellular	1.A.8.11.1		[79]
			H2Otx	H2O transport, peroxisomal	h2o[c] <=> h2o[x]	Transport, glyoxysome	1.A.8.11.1		[79]
			H2Otm	H2O transport, mitochondrial	h2o[c] <=> h2o[m]	Transport, mitochondria	1.A.8.11.1		[79]
			H2Othu	H2O transport by passive diffusion, Thylakoid Lumen	h2o[u] <=> h2o[h]	Transport, thylakoid lumen	1.A.8.11.1		[79]
genemark_Scaffold_7	0.006188586	3.391603054	HYDAh	(FeFe)-hydrogenase,	2 fdxrd[h] + 2 h[h]	Pyruvate	1.12.7.2	R0001	

Genes	Knocked-out genes flux value	Mutant/wildtype ratio	Reaction name in iCZ843	Reaction description	Formula	Subsystem	EC Number	KEGG ID	References
08-abinit-gene-0.19'				chloroplast	<=> 2 fdxox[h] + h2[h]	metabolism;Glyoxylate metabolism		9'	
			HYDA	(FeFe)-hydrogenase, cytoplasm	2 fdxrd[c] + 2 h[c] <=> 2 fdxox[c] + h2[c]	Pyruvate metabolism;Glyoxylate metabolism	1.12.7.2	R00019'	
			HYDAm	(FeFe)-hydrogenase, mitochondria	2 fdxrd[m] + 2 h[m] <=> 2 fdxox[m] + h2[m]	Pyruvate metabolism;Glyoxylate metabolism	1.12.7.2	R00019'	
'maker_Scaffold_1851-snap-gene-0.13'	0.006783179	3.717464849	FE3t	ferric iron uptake, plasma membrane	fe3[e] -> fe3[c]	Transport, extracellular	9.A.10.1.6;9.A.10.1.7		[79]
			FEROt	ferric iron updtake (oxidizing), plasma membrane	4 fe2[e] + 4 h[e] + o2[e] -> 4 fe3[c] + 2 h2o[e]	Transport, extracellular	9.A.10.1.5		[79]
			ASCBOR	L-ascorbate:oxygen oxidoreductase	2 ascb-L[u] + o2[u] -> 2 dhdascb[u] + 2 h2o[u]	Ascorbate and aldarate metabolism	1.10.3.3	'R00068'	
			UDPGLCURH	UDP-glucuronate glucuronohydrolase	h2o[c] + udpglcur[c] -> glcur[c] + h[c] + udp[c]	Ascorbate and aldarate metabolism	2.4.1.17	'R08615'	
'maker_Scaffold_313-snap-gene-0.153'	0.006783217	3.717485638	ASPth	Amino acid transporter (asp-L), chloroplast	asp-L[c] + h[c] <=> asp-L[h] + h[h]	Transport, chloroplast	2.A.18.6.6		[79]
			GLUth	Amino acid transporter	glu-L[h] + h[h] -> glu-	Transport,	2.A.18.6.6		[84]

Genes	Knocked-out genes flux value	Mutant/wildtype ratio	Reaction name in iCZ843	Reaction description	Formula	Subsystem	EC Number	KEGG ID	References
				(glu-L), chloroplast	L[c] + h[c]	chloroplast			
			ASPtx	Amino acid transporter (asp-L), glyoxysomal	asp-L[x] + h[x] <=> asp-L[c] + h[c]	Transport, glyoxysome	2.A.18.6.6		[84]
			ASPtm	Amino acid transporter (asp-L), mitochondrial	asp-L[c] + h[c] <=> asp-L[m] + h[m]	Transport, mitochondria	2.A.18.6.6		
			GLUtm	Amino acid transporter (glu-L), mitochondrial	glu-L[c] + h[c] <=> glu-L[m] + h[m]	Transport, mitochondria	2.A.18.6.6		
maker_Scaffold_458-augustus-gene-0.118'	0.007760304	4.252969986	GGDR	geranylgeranyl diphosphate reductase	ggdp[h] + 3 h[h] + 3 nadph[h] <=> 3 nadp[h] + pdp[h]	Biosynthesis of steroids	1.3.1.-	'R02063'	
			GGCHLDAR	geranylgeranyl-chlorophyllide a reductase	ggchlda[u] + 3 h[u] + 3 nadph[u] -> chla[u] + 3 nadp[u]	Porphyrin and chlorophyll metabolism	1.3.1.-		
			GGCHLDBR	geranylgeranyl-chlorophyllide b reductase	ggchldb[u] + 3 h[u] + 3 nadph[u] -> chlb[u] + 3 nadp[u]	Porphyrin and chlorophyll metabolism	1.3.1.-		
maker_Scaffold_969-augustus-gene-0.37'	0.007876128	4.316446334	ARGSS	argininosuccinate synthase	asp-L[c] + atp[c] + citr-L[c] -> amp[c] + argsuc[c] + 2 h[c] + ppi[c]	Arginine and proline metabolism; Urea cycle and metabolism of amino groups	6.3.4.5	'R01954'	

Genes	Knocked-out genes flux value	Mutant/wildtype ratio	Reaction name in iCZ843	Reaction description	Formula	Subsystem	EC Number	KEGG ID	References
maker_Scaffold_500-augustus-gene-0.34'	0.008119163	4.449639908	CDPDAGS18111Z160	CDP-diacylglycerol synthetase (18:1(11Z)/16:0)	ctp[c] + 3 h[c] + pa18111Z160[c] -> cdp12dgr18111Z160[c] + ppi[c]	Glycerophospholipid metabolism	2.7.7.41	'R01799'	
			CDPDAGS18111Z160h	CDP-diacylglycerol synthetase (18:1(11Z)/16:0), chloroplast	ctp[h] + 2 h[h] + pa18111Z160[h] -> cdp12dgr18111Z160[h] + ppi[h]	Glycerophospholipid metabolism	2.7.7.41	'R01799'	
			CDPDAGS1819Z160	CDP-diacylglycerol synthetase (18:1(9Z)/16:0)	ctp[c] + 3 h[c] + pa1819Z160[c] -> cdp12dgr1819Z160[c] + ppi[c]	Glycerophospholipid metabolism	2.7.7.41	'R01799'	
			CDPDAGS1819Z160h	CDP-diacylglycerol synthetase (18:1(9Z)/16:0), chloroplast	ctp[h] + 2 h[h] + pa1819Z160[h] -> cdp12dgr1819Z160[h] + ppi[h]	Glycerophospholipid metabolism	2.7.7.41	'R01799'	
genemark_Scaffold_211-abinit-gene-0.14'	0.008538892	4.67966903	ACOADAGAT16018111Z160	acyl-CoA: diacylglycerol acyltransferase (16:0)	12dgr16018111Z[c] + pmtcoa[c] -> coa[c] + tag16018111Z160[c]	Glycerolipid metabolism	2.3.1.20	'R02251'	
			ACOADAGAT16018111Z180	acyl-CoA: diacylglycerol acyltransferase (18:0)	12dgr16018111Z[c] + ocdccoa[c] -> coa[c] + tag16018111Z180[c]	Glycerolipid metabolism	2.3.1.20	'R02251'	
			ACOADAGAT16018111Z18111Z	acyl-CoA: diacylglycerol acyltransferase (18:1(11Z))	12dgr16018111Z[c] + ocdcecoa[c] -> coa[c] + tag16018111Z18111Z[	Glycerolipid metabolism	2.3.1.20	'R02251'	

Genes	Knocked-out genes flux value	Mutant/wildtype ratio	Reaction name in iCZ843	Reaction description	Formula	Subsystem	EC Number	KEGG ID	References
					c]				
			ACOADAGAT16018111Z1819Z	acyl-CoA: diacylglycerol acyltransferase (18:1(9Z))	12dgr16018111Z[c] + ocdce9coa[c] -> coa[c] + tag16018111Z1819Z[c]	Glycerolipid metabolism	2.3.1.20	'R02251'	
			ACOADAGAT16018111Z1835Z9Z12Z	acyl-CoA: diacylglycerol acyltransferase (18:3(5Z,9Z,12Z))	12dgr16018111Z[c] + pacoa[c] -> coa[c] + tag16018111Z1835Z9Z12Z[c]	Glycerolipid metabolism	2.3.1.20	'R02251'	
			ACOADAGAT16018111Z1845Z9Z12Z15Z	acyl-CoA: diacylglycerol acyltransferase (18:4(5Z,9Z,12Z,15Z))	12dgr16018111Z[c] + cacoa[c] -> coa[c] + tag16018111Z1845Z9Z12Z15Z[c]	Glycerolipid metabolism	2.3.1.20	'R02251'	
			ACOADAGAT161819Z160	acyl-CoA: diacylglycerol acyltransferase (16:0)	12dgr1601819Z[c] + pmtcoa[c] -> coa[c] + tag1601819Z160[c]	Glycerolipid metabolism	2.3.1.20	'R02251'	
			ACOADAGAT161819Z180	acyl-CoA: diacylglycerol acyltransferase (18:0)	12dgr1601819Z[c] + ocdccoa[c] -> coa[c] + tag1601819Z180[c]	Glycerolipid metabolism	2.3.1.20	'R02251'	
			ACOADAGAT161819Z18111Z	acyl-CoA: diacylglycerol acyltransferase (18:1(11Z))	12dgr1601819Z[c] + ocdcecoa[c] -> coa[c] + tag1601819Z18111Z[c]	Glycerolipid metabolism	2.3.1.20	'R02251'	
			ACOADAGAT160	acyl-CoA: diacylglycerol	12dgr1601819Z[c] +	Glycerolipid	2.3.1.20	'R02251'	

Genes	Knocked-out genes flux value	Mutant/wildtype ratio	Reaction name in iCZ843	Reaction description	Formula	Subsystem	EC Number	KEGG ID	References
			1819Z1819Z	acyltransferase (18:1(9Z))	ocdce9coa[c] -> coa[c] + tag1601819Z1819Z[c]	metabolism		1'	
			ACOADAGAT1601819Z1835Z9Z12Z	acyl-CoA: diacylglycerol acyltransferase (18:3(5Z,9Z,12Z))	12dgr1601819Z[c] + pacoa[c] -> coa[c] + tag1601819Z1835Z9Z12Z[c]	Glycerolipid metabolism	2.3.1.20	'R02251'	
			ACOADAGAT1601819Z1845Z9Z12Z15Z	acyl-CoA: diacylglycerol acyltransferase (18:4(5Z,9Z,12Z,15Z))	12dgr1601819Z[c] + cacoa[c] -> coa[c] + tag1601819Z1845Z9Z12Z15Z[c]	Glycerolipid metabolism	2.3.1.20	'R02251'	
			ACOADAGAT1801819Z160	acyl-CoA: diacylglycerol acyltransferase (16:0)	12dgr1801819Z[c] + pmtcoa[c] -> coa[c] + tag1801819Z160[c]	Glycerolipid metabolism	2.3.1.20	'R02251'	
			ACOADAGAT1801819Z180	acyl-CoA: diacylglycerol acyltransferase (18:0)	12dgr1801819Z[c] + ocdccoa[c] -> coa[c] + tag1801819Z180[c]	Glycerolipid metabolism	2.3.1.20	'R02251'	
			ACOADAGAT1801819Z18111Z	acyl-CoA: diacylglycerol acyltransferase (18:1(11Z))	12dgr1801819Z[c] + ocdcecoa[c] -> coa[c] + tag1801819Z18111Z[c]	Glycerolipid metabolism	2.3.1.20	'R02251'	
			ACOADAGAT1801819Z1819Z	acyl-CoA: diacylglycerol acyltransferase (18:1(9Z))	12dgr1801819Z[c] + ocdce9coa[c] -> coa[c] + tag1801819Z1819Z[c]	Glycerolipid metabolism	2.3.1.20	'R02251'	

Genes	Knocked-out genes flux value	Mutant/wildtype ratio	Reaction name in iCZ843	Reaction description	Formula	Subsystem	EC Number	KEGG ID	References
			ACOADAGAT1801819Z1835Z9Z12Z	acyl-CoA: diacylglycerol acyltransferase (18:3(5Z,9Z,12Z))	12dgr1801819Z[c] + pacoa[c] -> coa[c] + tag1801819Z1835Z9Z12Z[c]	Glycerolipid metabolism	2.3.1.20	'R02251'	
			ACOADAGAT1801819Z1845Z9Z12Z15Z	acyl-CoA: diacylglycerol acyltransferase (18:4(5Z,9Z,12Z,15Z))	12dgr1801819Z[c] + cacoa[c] -> coa[c] + tag1801819Z1845Z9Z12Z15Z[c]	Glycerolipid metabolism	2.3.1.20	'R02251'	
			ACOADAGAT18111Z18111Z160	acyl-CoA: diacylglycerol acyltransferase (16:0)	12dgr18111Z18111Z[c] + pmtcoa[c] -> coa[c] + tag18111Z18111Z160[c]	Glycerolipid metabolism	2.3.1.20	'R02251'	
			ACOADAGAT18111Z18111Z180	acyl-CoA: diacylglycerol acyltransferase (18:0)	12dgr18111Z18111Z[c] + ocdccoa[c] -> coa[c] + tag18111Z18111Z180[c]	Glycerolipid metabolism	2.3.1.20	'R02251'	
			ACOADAGAT18111Z18111Z18111Z	acyl-CoA: diacylglycerol acyltransferase (18:1(11Z))	12dgr18111Z18111Z[c] + ocdcecoa[c] -> coa[c] + tag18111Z18111Z18111Z[c]	Glycerolipid metabolism	2.3.1.20	'R02251'	
			ACOADAGAT18111Z18111Z1819Z	acyl-CoA: diacylglycerol acyltransferase (18:1(9Z))	12dgr18111Z18111Z[c] + ocdce9coa[c] -> coa[c] +	Glycerolipid metabolism	2.3.1.20	'R02251'	

Genes	Knocked-out genes flux value	Mutant/wildtype ratio	Reaction name in iCZ843	Reaction description	Formula	Subsystem	EC Number	KEGG ID	References
					tag18111Z18111Z1819Z[c]				
			ACOADAGAT18111Z18111Z1835Z9Z12Z	acyl-CoA: diacylglycerol acyltransferase (18:3(5Z,9Z,12Z))	12dgr18111Z18111Z[c] + pacoa[c] -> coa[c] + tag18111Z18111Z1835Z9Z12Z[c]	Glycerolipid metabolism	2.3.1.20	'R02251'	
			ACOADAGAT18111Z18111Z1845Z9Z12Z15Z	acyl-CoA: diacylglycerol acyltransferase (18:4(5Z,9Z,12Z,15Z))	12dgr18111Z18111Z[c] + cacoa[c] -> coa[c] + tag18111Z18111Z1845Z9Z12Z15Z[c]	Glycerolipid metabolism	2.3.1.20	'R02251'	
			ACOADAGAT18111Z1819Z160	acyl-CoA: diacylglycerol acyltransferase (16:0)	12dgr18111Z1819Z[c] + pmtcoa[c] -> coa[c] + tag18111Z1819Z160[c]	Glycerolipid metabolism	2.3.1.20	'R02251'	
			ACOADAGAT18111Z1819Z180	acyl-CoA: diacylglycerol acyltransferase (18:0)	12dgr18111Z1819Z[c] + ocdccoa[c] -> coa[c] + tag18111Z1819Z180[c]	Glycerolipid metabolism	2.3.1.20	'R02251'	
			ACOADAGAT18111Z1819Z18111Z	acyl-CoA: diacylglycerol acyltransferase (18:1(11Z))	12dgr18111Z1819Z[c] + ocdcecoa[c] -> coa[c] + tag18111Z1819Z1811	Glycerolipid metabolism	2.3.1.20	'R02251'	

Genes	Knocked-out genes flux value	Mutant/wildtype ratio	Reaction name in iCZ843	Reaction description	Formula	Subsystem	EC Number	KEGG ID	References
					1Z[c]				
			ACOADAGAT18111Z1819Z1819Z	acyl-CoA: diacylglycerol acyltransferase (18:1(9Z))	12dgr18111Z1819Z[c] + ocdce9coa[c] -> coa[c] + tag18111Z1819Z1819Z[c]	Glycerolipid metabolism	2.3.1.20	'R02251'	
			ACOADAGAT18111Z1819Z1835Z9Z12Z	acyl-CoA: diacylglycerol acyltransferase (18:3(5Z,9Z,12Z))	12dgr18111Z1819Z[c] + pacoa[c] -> coa[c] + tag18111Z1819Z1835Z9Z12Z[c]	Glycerolipid metabolism	2.3.1.20	'R02251'	
			ACOADAGAT18111Z1819Z1845Z9Z12Z15Z	acyl-CoA: diacylglycerol acyltransferase (18:4(5Z,9Z,12Z,15Z))	12dgr18111Z1819Z[c] + cacoa[c] -> coa[c] + tag18111Z1819Z1845Z9Z12Z15Z[c]	Glycerolipid metabolism	2.3.1.20	'R02251'	
			ACOADAGAT1819Z18111Z160	acyl-CoA: diacylglycerol acyltransferase (16:0)	12dgr1819Z18111Z[c] + pmtcoa[c] -> coa[c] + tag1819Z18111Z160[c]	Glycerolipid metabolism	2.3.1.20	'R02251'	
			ACOADAGAT1819Z18111Z180	acyl-CoA: diacylglycerol acyltransferase (18:0)	12dgr1819Z18111Z[c] + ocdccoa[c] -> coa[c] + tag1819Z18111Z180[c]	Glycerolipid metabolism	2.3.1.20	'R02251'	

Genes	Knocked-out genes flux value	Mutant/wildtype ratio	Reaction name in iCZ843	Reaction description	Formula	Subsystem	EC Number	KEGG ID	References
			ACOADAGAT1819Z18111Z18111Z	acyl-CoA: diacylglycerol acyltransferase (18:1(11Z))	12dgr1819Z18111Z[c] + ocdcecoa[c] -> coa[c] + tag1819Z18111Z18111Z[c]	Glycerolipid metabolism	2.3.1.20	'R02251'	
			ACOADAGAT1819Z18111Z1819Z	acyl-CoA: diacylglycerol acyltransferase (18:1(9Z))	12dgr1819Z18111Z[c] + ocdce9coa[c] -> coa[c] + tag1819Z18111Z1819Z[c]	Glycerolipid metabolism	2.3.1.20	'R02251'	
			ACOADAGAT1819Z18111Z1835Z9Z12Z	acyl-CoA: diacylglycerol acyltransferase (18:3(5Z,9Z,12Z))	12dgr1819Z18111Z[c] + pacoa[c] -> coa[c] + tag1819Z18111Z1835Z9Z12Z[c]	Glycerolipid metabolism	2.3.1.20	'R02251'	
			ACOADAGAT1819Z18111Z1845Z9Z12Z15Z	acyl-CoA: diacylglycerol acyltransferase (18:4(5Z,9Z,12Z,15Z))	12dgr1819Z18111Z[c] + cacoa[c] -> coa[c] + tag1819Z18111Z1845Z9Z12Z15Z[c]	Glycerolipid metabolism	2.3.1.20	'R02251'	
			ACOADAGAT1819Z1819Z160	acyl-CoA: diacylglycerol acyltransferase (16:0)	12dgr1819Z1819Z[c] + pmtcoa[c] -> coa[c] + tag1819Z1819Z160[c]	Glycerolipid metabolism	2.3.1.20	'R02251'	
			ACOADAGAT1819Z1819Z180	acyl-CoA: diacylglycerol acyltransferase (18:0)	12dgr1819Z1819Z[c] + ocdccoa[c] ->	Glycerolipid metabolism	2.3.1.20	'R02251'	

Genes	Knocked-out genes flux value	Mutant/wildtype ratio	Reaction name in iCZ843	Reaction description	Formula	Subsystem	EC Number	KEGG ID	References
					coa[c] + tag1819Z1819Z180[c]				
			ACOADAGAT1819Z1819Z18111Z	acyl-CoA: diacylglycerol acyltransferase (18:1(11Z))	12dgr1819Z1819Z[c] + ocdcecoa[c] -> coa[c] + tag1819Z1819Z18111Z[c]	Glycerolipid metabolism	2.3.1.20	'R02251'	
			ACOADAGAT1819Z1819Z1819Z	acyl-CoA: diacylglycerol acyltransferase (18:1(9Z))	12dgr1819Z1819Z[c] + ocdce9coa[c] -> coa[c] + tag1819Z1819Z1819Z[c]	Glycerolipid metabolism	2.3.1.20	'R02251'	
			ACOADAGAT1819Z1819Z1835Z9Z12Z	acyl-CoA: diacylglycerol acyltransferase (18:3(5Z,9Z,12Z))	12dgr1819Z1819Z[c] + pacoa[c] -> coa[c] + tag1819Z1819Z1835Z9Z12Z[c]	Glycerolipid metabolism	2.3.1.20	'R02251'	
			ACOADAGAT1819Z1819Z1845Z9Z12Z15Z	acyl-CoA: diacylglycerol acyltransferase (18:4(5Z,9Z,12Z,15Z))	12dgr1819Z1819Z[c] + cacoa[c] -> coa[c] + tag1819Z1819Z1845Z9Z12Z15Z[c]	Glycerolipid metabolism	2.3.1.20	'R02251'	
genemark_Scaffold_484-abinit-gene-0.7'	0.00932576	5.110905501	PDHam1hi	pyruvate dehydrogenase (acetyl-transferring)	h[h] + pyr[h] + thmpp[h] -> 2ahethmpp[h] + co2[h]	Glycolysis / Gluconeogenesis;Alanine and aspartate	1.2.4.1;2.2.1.6;4.1.1.1	'R00014'	[91]

Genes	Knocked-out genes flux value	Mutant/wildtype ratio	Reaction name in iCZ843	Reaction description	Formula	Subsystem	EC Number	KEGG ID	References
						metabolism			
			PDHam2hi	Pyruvate dehydrogenase (lipoamide), chloroplast irreversible	2ahethmpp[h] + lpam[h] -> adhlam[h] + thmpp[h]	Glycolysis / Gluconeogenesis;Alanine and aspartate metabolism	1.2.4.1;2.2.1.6;4.1.1.1	'R03270'	[91]
'maker_Scaffold_176-augustus-gene-0.194'	0.00932576	5.110905501	PDHam1hi	pyruvate dehydrogenase (acetyl-transferring)	h[h] + pyr[h] + thmpp[h] -> 2ahethmpp[h] + co2[h]	Glycolysis / Gluconeogenesis;Alanine and aspartate metabolism	1.2.4.1;2.2.1.6;4.1.1.1	'R00014'	[91]
			PDHam2hi	Pyruvate dehydrogenase (lipoamide), chloroplast irreversible	2ahethmpp[h] + lpam[h] -> adhlam[h] + thmpp[h]	Glycolysis / Gluconeogenesis;Alanine and aspartate metabolism	1.2.4.1;2.2.1.6;4.1.1.1	'R03270'	[91]
'maker_Scaffold_1306-snap-gene-0.47'	0.009482098	5.196585076	BCTA(val)m	branched-chain-amino-acid transaminase, valine forming, mitochondria	3mob[m] + glu-L[m] <=> akg[m] + val-L[m]	Pantothenate and CoA biosynthesis	2.6.1.42	'R01214'	
			BCTA	branched-chain-amino-acid transaminase	3mop[c] + glu-L[c] <=> akg[c] + ile-L[c]	Valine, leucine and isoleucine degradation;Valine, leucine and	2.6.1.42	'R02199'	

Genes	Knocked-out genes flux value	Mutant/wildtype ratio	Reaction name in iCZ843	Reaction description	Formula	Subsystem	EC Number	KEGG ID	References
						isoleucine biosynthesis			
			BCTA(glu)	branched-chain-amino-acid transaminase, glutamate forming	akg[c] + leu-L[c] <=> 4mop[c] + glu-L[c]	Valine, leucine and isoleucine degradation;Valine, leucine and isoleucine biosynthesis	2.6.1.42	'R01090'	
			BCTA(glu)h	branched-chain-amino-acid transaminase, glutamate forming, chloroplast	akg[h] + leu-L[h] <=> 4mop[h] + glu-L[h]	Valine, leucine and isoleucine degradation;Valine, leucine and isoleucine biosynthesis	2.6.1.42	'R01090'	
			BCTA(val)	branched-chain-amino-acid transaminase, valine forming	akg[c] + val-L[c] -> 3mob[c] + glu-L[c]	Valine, leucine and isoleucine degradation;Valine, leucine and isoleucine biosynthesis	2.6.1.42	'R01214'	

Genes	Knocked-out genes flux value	Mutant/wildtype ratio	Reaction name in iCZ843	Reaction description	Formula	Subsystem	EC Number	KEGG ID	References
			BCTA(val)h	branched-chain-amino-acid transaminase, valine forming, chloroplast	akg[h] + val-L[h] -> 3mob[h] + glu-L[h]	Valine, leucine and isoleucine degradation;Valine, leucine and isoleucine biosynthesis	2.6.1.42	'R01214'	
			BCTAh	branched-chain-amino-acid transaminase, chloroplast	3mop[h] + glu-L[h] <=> akg[h] + ile-L[h]	Valine, leucine and isoleucine degradation;Valine, leucine and isoleucine biosynthesis	2.6.1.42	'R02199'	
maker_Scaffold_859-augustus-gene-0.55'	0.009501374	5.207149276	SERDC	serine decarboxylase	h[c] + ser-L[c] -> co2[c] + etha[c]	Glycerophospholipid metabolism	4.1.1.22		
			HDC	histidine decarboxylase	2 h[c] + his-L[c] -> co2[c] + hista[c]	Histidine metabolism	4.1.1.22	'R01167'	
'maker_Scaffold_97-snap-gene-0.144'	0.009546778	5.232032156	GLU4ABUTthi	Glutamate:gamma-aminobutyrate antiporter, chloroplast irreversible	4abut[c] + glu-L[h] -> 4abut[h] + glu-L[c]	Transport, chloroplast	2.A.3.7.1		[84]
			GLUNA1th	Glutamate:Na+	glu-L[h] + na1[h] ->	Transport,	2.A.23.2.2		[84]

Genes	Knocked-out genes flux value	Mutant/wildtype ratio	Reaction name in iCZ843	Reaction description	Formula	Subsystem	EC Number	KEGG ID	References
				symporter, chloroplast	glu-L[c] + na1[c]	chloroplast			
			GLYth	Amino acid transporter (gly), chloroplast	gly[c] + h[c] <=> gly[h] + h[h]	Transport, chloroplast	2.A.3.3.3		[84]
			HISth	Amino acid transporter (his-L), chloroplast	h[c] + his-L[c] <=> h[h] + his-L[h]	Transport, chloroplast	2.A.3.3.3		[84]
			SERth	Amino acid transporter (ser-L), chloroplast	h[c] + ser-L[c] <=> h[h] + ser-L[h]	Transport, chloroplast	2.A.3.3.3		[84]
			THRth	Amino acid transporter (thr-L), chloroplast	h[c] + thr-L[c] <=> h[h] + thr-L[h]	Transport, chloroplast	2.A.3.3.3		[84]
			VALth	Amino acid transporter (val-L), chloroplast	h[c] + val-L[c] <=> h[h] + val-L[h]	Transport, chloroplast	2.A.3.3.3		[84]
			HISt	Amino acid transporter (his-L), extracellular	h[e] + his-L[e] <=> h[c] + his-L[c]	Transport, extracellular	2.A.3.3.3		[84]
			ALAtx	Amino acid transporter (ala-L), glyoxysomal	ala-L[c] + h[c] <=> ala-L[x] + h[x]	Transport, glyoxysome	2.A.3.3.3		[84]
			GLYtx	Amino acid transporter (gly), glyoxysomal	gly[c] + h[c] <=> gly[x] + h[x]	Transport, glyoxysome	2.A.3.3.3		[84]
			ALAtm	Amino acid transporter (ala-L), mitochondrial	ala-L[c] + h[c] <=> ala-L[m] + h[m]	Transport, mitochondria	2.A.3.3.3		[84]
			GLYtm	Amino acid transporter (gly), mitochondrial	gly[c] + h[c] <=> gly[m] + h[m]	Transport, mitochondria	2.A.3.3.3		[84]
			SERtm	Amino acid transporter (ser-L), mitochondrial	h[c] + ser-L[c] <=> h[m] + ser-L[m]	Transport, mitochondria	2.A.3.3.3		[84]
			THRtm	Amino acid transporter (thr-L), mitochondrial	h[c] + thr-L[c] <=> h[m] + thr-L[m]	Transport, mitochondria	2.A.3.3.3		[84]
			VALtm	Amino acid transporter	h[c] + val-L[c] <=>	Transport,	2.A.3.3.3		[84]

Genes	Knocked-out genes flux value	Mutant/wildtype ratio	Reaction name in iCZ843	Reaction description	Formula	Subsystem	EC Number	KEGG ID	References
				(val-L), mitochondrial	h[m] + val-L[m]	mitochondria			
'maker_Scaffold_23-augustus-gene-0.68'	0.009623209	5.273919978	HBCHLm	3-Hydroxybutanoyl-CoA hydro-lyase, mitochondria	3hbcoa[m] <=> b2coa[m] + h2o[m]	Butanoate metabolism	4.2.1.17;5.3.3.8	'R03026'	
			ECOAH2m	enoyl-CoA hydratase (C6:0)	3hhcoa[m] <=> h2o[m] + hx2coa[m]	Fatty acid elongation in mitochondria	4.2.1.17	'R04749'	
			ECOAH3m	enoyl-CoA hydratase (C8:0)	3hocoa[m] <=> h2o[m] + oc2coa[m]	Fatty acid elongation in mitochondria	4.2.1.17	'R04746'	
			ECOAH4m	enoyl-CoA hydratase (C10:0)	3hdcoa[m] <=> dc2coa[m] + h2o[m]	Fatty acid elongation in mitochondria	4.2.1.17	'R04744'	
			ECOAH5m	enoyl-CoA hydratase (C12:0)	3hddcoa[m] <=> dd2coa[m] + h2o[m]	Fatty acid elongation in mitochondria	4.2.1.17	'R04170'	
			ECOAH6m	enoyl-CoA hydratase (C14:0)	3htdcoa[m] <=> h2o[m] + td2coa[m]	Fatty acid elongation in mitochondria	4.2.1.17	'R04740'	
			ECOAH7m	enoyl-CoA hydratase (C16:0)	3hhdcoa[m] <=> h2o[m] + hdd2coa[m]	Fatty acid elongation in mitochondria	4.2.1.17	'R04738'	
			HPHLm	3-Hydroxypropionyl-CoA hydro-lyase, mitochondria	3hpcoa[m] <=> h2o[m] + prpncoa[m]	Propanoate metabolism	4.2.1.17;5.3.3.8	'R03045'	
			ECH(3hibutcoa)	enoyl-coa hydratase, 3-	2mp2coa[m] +	Valine,	4.2.1.17	'R0422	

Genes	Knocked-out genes flux value	Mutant/wildtype ratio	Reaction name in iCZ843	Reaction description	Formula	Subsystem	EC Number	KEGG ID	References
				Hydroxyisobutyryl-CoA forming	h2o[m] -> 3hibutcoa[m]	leucine and isoleucine degradation		4'	
			ECH(3hivcoa)	enoyl-coa hydratase, 3-Hydroxyisovaleryl-CoA forming	3mb2coa[m] + h2o[m] <=> 3hivcoa[m]	Valine, leucine and isoleucine degradation	4.2.1.17	'R0413 7'	
			ECH(3hmbcoa)	enoyl-coa hydratase, 3-Hydroxy-2-methylbutyryl-CoA forming	2mb2coa[m] + h2o[m] -> 3hmbcoa[m]	Valine, leucine and isoleucine degradation	4.2.1.17	'R0420 4'	
'maker_Scaffold_801-augustus-gene-0.50'	0.01001376	5.487958113	GLYS	glycine synthase	co2[m] + mlthf[m] + nadh[m] + nh4[m] -> gly[m] + nad[m] + thf[m]	Nitrogen metabolism	2.1.2.10	'R0122 1'	
			MTAM	5,10-Methenyltetrahydrofolate aminomethyltransferase	5fthf[m] + 2 h[m] -> h2o[m] + methf[m]	One carbon pool by folate	2.1.2.10	'R0230 0'	
			MTAM(nh4)	5,10-Methenyltetrahydrofolate aminomethyltransferase (ammonia-forming)	alpro[m] + h[m] + thf[m] <=> dhlpro[m] + mlthf[m] + nh4[m]	One carbon pool by folate	2.1.2.10	'R0412 5'	
maker_Scaffold_126-snap-gene-0.176'	0.010104814	5.537859341	ICDH	isocitrate dehydrogenase (NADP)	icit[c] + nadp[c] <=> akg[c] + co2[c] +	TCA cycle;CO2	1.1.1.42	'R0026 7'	[92]

Genes	Knocked-out genes flux value	Mutant/wildtype ratio	Reaction name in iCZ843	Reaction description	Formula	Subsystem	EC Number	KEGG ID	References
					nadph[c]	fixation			
			ICDHhr	Isocitrate dehydrogenase (NADP+), reversible	icit[h] + nadp[h] <=> akg[h] + co2[h] + nadph[h]	TCA cycle;CO2 fixation	1.1.1.42	'R00267'	
genemark_Scaffold_185-abinit-gene-0.3'	0.010133586	5.553627655	TAh	transaldolase	g3p[h] + s7p[h] <=> e4p[h] + f6p-B[h]	Pentose phosphate pathway	2.2.1.2	'R01827'	[93]
augustus_masked_Scaffold_1605-abinit-gene-0.4'	0.010138754	5.556460194	STARCH300DEGR2A	degradation of starch300 by phosphorylase, amylase, dextrinase, maltase (chloroplast), 1:5 glc-A:g1p (dark-dep rxn)	49 h2o[h] + 250 pi[h] + starch300[h] -> 250 g1p[h] + 50 glc-A[h]	Starch metabolism	2.4.1.1 + 3.2.1.1 + 3.2.1.2 + 3.2.1.142 + 3.2.1.20	'R02111 + R02108 + R02112 + R00028'	[94, 95]
			STARCH300DEGR2B	degradation of starch300 by phosphorylase, amylase, dextrinase, maltase (chloroplast), 1:5 glc-B:g1p (dark-dep rxn)	49 h2o[h] + 250 pi[h] + starch300[h] -> 250 g1p[h] + 50 glc-B[h]	Starch metabolism	2.4.1.1 + 3.2.1.1 + 3.2.1.2 + 3.2.1.142 + 3.2.1.20	'R02111 + R02108 + R02112 + R00028'	[94, 95]
			STARCH300DEGRA	degradation of starch300 by phosphorylase,	74 h2o[h] + 225 pi[h] + starch300[h] -> 225 g1p[h] + 75 glc-A[h]	Starch metabolism	2.4.1.1 + 3.2.1.1 + 3.2.1.2 +	'R02111 + R0210	[94, 95]

Genes	Knocked-out genes flux value	Mutant/wildtype ratio	Reaction name in iCZ843	Reaction description	Formula	Subsystem	EC Number	KEGG ID	References
				amylase, dextrinase, maltase (chloroplast), 1:3 glc-A:g1p (light-dep rxn)			3.2.1.142 + 3.2.1.20	8 + R0211 2 + R0002 8'	
			STARCH300DEGRB	degradation of starch300 by phosphorylase, amylase, dextrinase, maltase (chloroplast), 1:3 glc-B:g1p (light-dep rxn)	74 h2o[h] + 225 pi[h] + starch300[h] -> 225 g1p[h] + 75 glc-B[h]	Starch metabolism	2.4.1.1 + 3.2.1.1 + 3.2.1.2 + 3.2.1.142 + 3.2.1.20	'R0211 1 + R0210 8 + R0211 2 + R0002 8'	[94, 95]
maker_Scaffold_1153-augustus-gene-0.48'	0.010138754	5.556460194	STARCH300DEGR2A	degradation of starch300 by phosphorylase, amylase, dextrinase, maltase (chloroplast), 1:5 glc-A:g1p (dark-dep rxn)	49 h2o[h] + 250 pi[h] + starch300[h] -> 250 g1p[h] + 50 glc-A[h]	Starch metabolism	2.4.1.1 + 3.2.1.1 + 3.2.1.2 + 3.2.1.142 + 3.2.1.20	'R0211 1 + R0210 8 + R0211 2 + R0002 8'	[94, 95]
			STARCH300DEGR2B	degradation of starch300 by phosphorylase, amylase, dextrinase, maltase (chloroplast),	49 h2o[h] + 250 pi[h] + starch300[h] -> 250 g1p[h] + 50 glc-B[h]	Starch metabolism	2.4.1.1 + 3.2.1.1 + 3.2.1.2 + 3.2.1.142 + 3.2.1.20	'R0211 1 + R0210 8 + R0211	[94, 95]

Genes	Knocked-out genes flux value	Mutant/wildtype ratio	Reaction name in iCZ843	Reaction description	Formula	Subsystem	EC Number	KEGG ID	References
				1:5 glc-B:g1p (dark-dep rxn)				2 + R00028'	
			STARCH300DEGRA	degradation of starch300 by phosphorylase, amylase, dextrinase, maltase (chloroplast), 1:3 glc-A:g1p (light-dep rxn)	74 h2o[h] + 225 pi[h] + starch300[h] -> 225 g1p[h] + 75 glc-A[h]	Starch metabolism	2.4.1.1 + 3.2.1.1 + 3.2.1.2 + 3.2.1.142 + 3.2.1.20	'R02111 + R02108 + R02112 + R00028'	[94, 95]
			STARCH300DEGRB	degradation of starch300 by phosphorylase, amylase, dextrinase, maltase (chloroplast), 1:3 glc-B:g1p (light-dep rxn)	74 h2o[h] + 225 pi[h] + starch300[h] -> 225 g1p[h] + 75 glc-B[h]	Starch metabolism	2.4.1.1 + 3.2.1.1 + 3.2.1.2 + 3.2.1.142 + 3.2.1.20	'R02111 + R02108 + R02112 + R00028'	[94, 95]
'genemark_Scaffold_1220-abinit-gene-0.13'	0.010141318	5.557864857	ACOA100OR	Decanoyl-CoA:(acceptor) 2,3-oxidoreductase	dcacoa[x] + fad[x] -> dc2coa[x] + fadh2[x]	Fatty acid metabolism	1.3.99.-;1.3.3.6	'R04754'	
			ACOA120OR	Lauroyl-CoA:(acceptor) 2,3-oxidoreductase	ddcacoa[x] + fad[x] -> dd2coa[x] + fadh2[x]	Fatty acid metabolism	1.3.99.-;1.3.3.6	'R03857'	
			ACOA140OR	Tetradecanoyl-	fad[x] + tdcoa[x] ->	Fatty acid	1.3.99.-	'R0399	

Genes	Knocked-out genes flux value	Mutant/wildtype ratio	Reaction name in iCZ843	Reaction description	Formula	Subsystem	EC Number	KEGG ID	References
				CoA:(acceptor) 2,3-oxidoreductase	fadh2[x] + td2coa[x]	metabolism	;1.3.3.6	0'	
			ACOA160OR	Palmitoyl-CoA:oxygen 2-oxidoreductase	fad[x] + pmtcoa[x] -> fadh2[x] + hdd2coa[x]	Fatty acid metabolism	1.3.99.-;1.3.3.6	'R0127 9'	
			ACOA40OR	Butanoyl-CoA:oxygen 2-oxidoreductase	btcoa[x] + fad[x] -> b2coa[x] + fadh2[x]	Fatty acid metabolism	1.3.99.-;1.3.99.2; 1.3.3.6	'R0117 5'	
			ACOA60OR	Hexanoyl-CoA:(acceptor) 2,3-oxidoreductase	fad[x] + hxcoa[x] -> fadh2[x] + hx2coa[x]	Fatty acid metabolism	1.3.99.-;1.3.99.2; 1.3.3.6	'R0475 1'	
			ACOA80OR	Octanoyl-CoA:oxygen 2-oxidoreductase	fad[x] + occoa[x] -> fadh2[x] + oc2coa[x]	Fatty acid metabolism	1.3.99.-;1.3.3.6	'R0377 7'	
			PCNO	propanoyl-CoA:NADP+ 2-oxidoreductase	nadp[c] + ppcoa[c] <=> h[c] + nadph[c] + prpncoa[c]	Propanoate metabolism	1.3.1.-	'R0091 9'	
			OPC8COAOR	OPC-8:0 CoA:oxygen 2-oxidoreductase	fad[c] + opc8coa[c] -> fadh2[c] + opc8coat2e[c]	alpha-Linolenic acid metabolism	1.3.3.6	'R0788 8'	[96, 97]
			OPC6COAOR	OPC-6:0 CoA:oxygen 2-oxidoreductase	fad[c] + opc6coa[c] -> fadh2[c] + opc6coat2e[c]	alpha-Linolenic acid metabolism	1.3.3.6	'R0789 2'	[96, 97]
			OPC4COAOR	OPC-4:0 CoA:oxygen 2-oxidoreductase	fad[c] + opc4coa[c] -> fadh2[c] + opc4coat2e[c]	alpha-Linolenic acid metabolism	1.3.3.6	'R0789 6'	[96, 97]
maker_Scaffold_145-augustus-gene-0.53'	0.01014138	5.557898894	GLYDH	Glycerate dehydrogenase	h[c] + hpyr[c] + nadh[c] -> glyc-R[c] +	Glyoxylate metabolism	1.1.1.29	'R0138 8'	

Genes	Knocked-out genes flux value	Mutant/wildtype ratio	Reaction name in iCZ843	Reaction description	Formula	Subsystem	EC Number	KEGG ID	References
					nad[c]				
			glycltdhCv	Glycerate dehydrogenase, chloroplast	glx[h] + h[h] + nadh[h] -> glyclt[h] + nad[h]	Glyoxylate and dicarboxylate metabolism	1.1.1.29	'R00717'	[98]
			glydhhCv	Glycerate dehydrogenase, chloroplast	h[h] + nadh[h] + hpyr[h] -> glyc-R[h] + nad[h]	Glyoxylate and dicarboxylate metabolism	1.1.1.29	'R01388'	[98]
maker_Scaffold_439-snap-gene-0.203'	0.01014138	5.557899113	THRAL	threonine ammonia-lyase (serine)	ser-L[h] <=> nh4[h] + pyr[h]	Glycine, serine and threonine metabolism	4.3.1.19	'R00220'	
			TAL	threonine ammonia-lyase	thr-L[h] -> 2obut[h] + nh4[h]	Valine, leucine and isoleucine biosynthesis	4.3.1.19	'R00996'	
genemark_Scaffold_1016-abinit-gene-0.16'	0.014709455	8.061394724	ETFQO	Electron transfer flavoprotein-ubiquinone oxidoreductase	etfrd[m] + q8[m] -> etfox[m] + q8h2[m]	Cofactor recycling	1.5.5.1	'R04433'	

APPENDIX B

APPENDIX B 12 potential gene after single gene deletion using LMOMA, Photoautotrophy

Genes	Knocked-out genes flux value	Mutant/wildtype ratio	Reaction name in iCZ843	Reaction description	Formula	Subsystem	EC Number	KEGG ID	References
genemark_Scaffold_651-abinit-gene-0.33'	0.002087133	1.023273216	ADPA	ADP-apyrase	adp[c] + h2o[c] -> amp[c] + h[c] + pi[c]	Purine metabolism	3.6.1.5	'R00122'	
			ATPPH	ATP phosphohydrolase	atp[c] + h2o[c] -> adp[c] + h[c] + pi[c]	Purine metabolism	3.6.1.5;3.6.1.3;3.6.1.15	'R00086'	
			ATPPHm	ATP phosphohydrolase, mitochondria	atp[m] + h2o[m] -> adp[m] + h[m] + pi[m]	Purine metabolism	3.6.1.5;3.6.1.3;3.6.1.15	'R00086'	
			GDPA	GDP-apyrase	gdp[c] + h2o[c] -> gmp[c] + h[c] + pi[c]	Purine metabolism	3.6.1.5	'R00328'	
			GTPA	GTP-apyrase	gtp[c] + h2o[c] -> gdp[c] + h[c] + pi[c]	Purine metabolism	3.6.1.5	'R00335'	
			IDPA	IDP-apyrase	h2o[c] + idp[c] -> h[c] + imp[c] + pi[c]	Purine metabolism	3.6.1.5	'R00961'	
			ITPA	ITP-apyrase	h[c] + idp[c] + pi[c] -> h2o[c] + itp[c]	Purine metabolism	3.6.1.5	'R00719'	
			CDPPH	CDP phosphohydrolase	cdp[c] + h2o[c] -> cmp[c] + h[c] + pi[c]	Pyrimidine metabolism	3.6.1.5	'R00514'	
			CTPH	CTP phosphohydrolase	ctp[c] + h2o[c] -> cdp[c] + h[c] + pi[c]	Pyrimidine metabolism	3.6.1.5	'R00569'	
			DTNH	dTTP nucleotidohydrolase	dttp[c] + h2o[c] -> dtdp[c] + h[c] + pi[c]	Pyrimidine metabolism	3.6.1.5	'R02095'	

			DTPH	dTDP phosphohydrolase	dtdp[c] + h2o[c] -> dtmp[c] + h[c] + pi[c]	Pyrimidine metabolism	3.6.1.5	'R02092'
			UPH	UDP phosphohydrolase	h2o[c] + udp[c] -> h[c] + pi[c] + ump[c]	Pyrimidine metabolism	3.6.1.5	'R00155'
			UTPH	UTP phosphohydrolase	h2o[c] + utp[c] -> h[c] + pi[c] + udp[c]	Pyrimidine metabolism	3.6.1.5	'R00159'
genemark_Scaffold_335-abinit-gene-0.8'	0.002284596	1.120085158	MAGAH160	1-acylglycerol acylhydrolase (16:0)	h2o[c] + mag160[c] -> glyc[c] + h[c] + hdca[c]	Glycerolipid metabolism	3.1.1.23	'R01351'
			MAGAH180	1-acylglycerol acylhydrolase (18:0)	h2o[c] + mag180[c] -> glyc[c] + h[c] + ocdca[c]	Glycerolipid metabolism	3.1.1.23	'R01351'
			MAGAH18111Z	1-acylglycerol acylhydrolase (18:1(11Z))	h2o[c] + mag18111Z[c] -> glyc[c] + h[c] + ocdcea[c]	Glycerolipid metabolism	3.1.1.23	'R01351'
			MAGAH1819Z	1-acylglycerol acylhydrolase (18:1(9Z))	h2o[c] + mag1819Z[c] -> glyc[c] + h[c] + ocdce9a[c]	Glycerolipid metabolism	3.1.1.23	'R01351'
			DHR	dihydroorotase	cbasp[m] <=> dhor-S[m] + h[m] + h2o[m]	Pyrimidine metabolism	3.5.2.3	'R01993'
maker_Scaffold_1801-augustus-gene-0.22'	0.002284596	1.120085158	AACP1819ZS	long-chain-fatty-acid-[acyl-carrier-protein] ligase (18:1(9Z))	ACP[h] + atp[h] + ocdce9a[h] -> amp[h] + h[h] + octe9ACP[h] + ppi[h]	Fatty acid metabolism	6.2.1.20	'R01406'
			AACPS1	long-chain-fatty-acid-[acyl-carrier-protein] ligase (14:0)	ACP[h] + atp[h] + ttdca[h] -> amp[h] + h[h] + myrsACP[h] + ppi[h]	Fatty acid metabolism	6.2.1.20	'R01406'
			AACPS3	long-chain-fatty-acid-	ACP[h] + atp[h] +	Fatty acid	6.2.1.20	'R01406

	[acyl-carrier-protein] ligase (16:0)	hdca[h] -> amp[h] + h[h] + palmACP[h] + ppi[h]	metabolism		'
AACPS4	long-chain-fatty-acid-[acyl-carrier-protein] ligase (16:1(9Z))	ACP[h] + atp[h] + hdcea[h] -> amp[h] + h[h] + hdeACP[h] + ppi[h]	Fatty acid metabolism	6.2.1.20	'R01406 '
AACPS5	long-chain-fatty-acid-[acyl-carrier-protein] ligase (18:1(11Z))	ACP[h] + atp[h] + ocdcea[h] -> amp[h] + h[h] + octeACP[h] + ppi[h]	Fatty acid metabolism	6.2.1.20	'R01406 '
AACPS6	long-chain-fatty-acid-[acyl-carrier-protein] ligase (18:0)	ACP[h] + atp[h] + ocdca[h] -> amp[h] + h[h] + ocdcaACP[h] + ppi[h]	Fatty acid metabolism	6.2.1.20	'R01406 '
AGPAT1601819Z h	1-hexadecanoyl-sn-glycerol 3-phosphate O-acyltransferase ((9Z)-C18:1) (ACP substrate)	1hdecg3p[h] + octe9ACP[h] -> ACP[h] + pa1601819Z[h]	Glycerolipid metabolism	2.3.1.51	'R02241 '
AGPAT160h	1-hexadecanoyl-sn-glycerol 3-phosphate O-acyltransferase (n-C16:0) (ACP substrate)	1hdecg3p[h] + palmACP[h] -> ACP[h] + pa160[h]	Glycerolipid metabolism	2.3.1.51	'R02241 '
AGPAT1801819Z h	1-octadecanoyl-sn-glycerol 3-phosphate O-acyltransferase ((9Z)-C18:1) (ACP substrate)	1odecg3p[h] + octe9ACP[h] -> ACP[h] + pa1801819Z[h]	Glycerolipid metabolism	2.3.1.51	'R02241 '
AGPAT18111Z16	1-octadec-11-enoyl-sn-	1odec11eg3p[h] +	Glycerolipid	2.3.1.51	'R02241

0h	glycerol 3-phosphate O-acyltransferase (n-C16:0) (ACP substrate)	palmACP[h] -> ACP[h] + pa18111Z160[h]	metabolism		'	
AGPAT18111Z1819Zh	1-octadec-11-enoyl-sn-glycerol 3-phosphate O-acyltransferase ((9Z)-C18:1) (ACP substrate)	1odec11eg3p[h] + octe9ACP[h] -> ACP[h] + pa18111Z1819Z[h]	Glycerolipid metabolism	2.3.1.51	'R02241 '	
AGPAT1819Z160h	1-octadec-9-enoyl-sn-glycerol 3-phosphateO-acyltransferase (n-C16:0) (ACP substrate)	1odec9eg3p[h] + palmACP[h] -> ACP[h] + pa1819Z160[h]	Glycerolipid metabolism	2.3.1.51	'R02241 '	
AGPAT1819Z1619Zh	1-octadec-9-enoyl-sn-glycerol 3-phosphateO-acyltransferase ((9Z)-C16:1) (ACP substrate)	1odec9eg3p[h] + hdeACP[h] -> ACP[h] + pa1819Z1619Z[h]	Glycerolipid metabolism	2.3.1.51	'R02241 '	
AGPAT1819Z18111Zh	1-octadec-9-enoyl-sn-glycerol 3-phosphateO-acyltransferase ((11Z)-C18:1) (ACP substrate)	1odec9eg3p[h] + octeACP[h] -> ACP[h] + pa1819Z18111Z[h]	Glycerolipid metabolism	2.3.1.51	'R02241 '	
AGPAT1819Z1819Zh	1-octadec-9-enoyl-sn-glycerol 3-phosphateO-acyltransferase ((9Z)-C18:1) (ACP substrate)	1odec9eg3p[h] + octe9ACP[h] -> ACP[h] + pa1819Z1819Z[h]	Glycerolipid metabolism	2.3.1.51	'R02241 '	
G3PAT160	glycerol-3-phosphate: acyl-coa acyltransferase (16:0)	glyc3p[c] + pmtcoa[c] -> 1hdecg3p[c] + coa[c]	Glycerolipid metabolism	2.3.1.15	'R00851 '	[87, 88]
G3PAT160h	glycerol-3-phosphate acyltransferase (C16:0)	glyc3p[h] + palmACP[h] ->	Glycerolipid metabolism	2.3.1.15	'R00851 '	[87, 88]

					1hdecg3p[h] + ACP[h]				
			G3PAT180	glycerol-3-phosphate: acyl-coa acyltransferase (18:0)	glyc3p[c] + ocdccoa[c] -> 1odecg3p[c] + coa[c]	Glycerolipid metabolism	2.3.1.15	'R00851'	[87, 88]
			G3PAT180h	glycerol-3-phosphate acyltransferase (C18:0)	glyc3p[h] + ocdcaACP[h] -> 1odecg3p[h] + ACP[h]	Glycerolipid metabolism	2.3.1.15	'R00851'	[87, 88]
			G3PAT181	glycerol-3-phosphate: acyl-coa acyltransferase (18:1(11Z))	glyc3p[c] + ocdcecoa[c] -> 1odec11eg3p[c] + coa[c]	Glycerolipid metabolism	2.3.1.15	'R00851'	[87, 88]
			G3PAT1819Z	glycerol-3-phosphate: acyl-coa acyltransferase (18:1(9Z))	glyc3p[c] + ocdce9coa[c] -> 1odec9eg3p[c] + coa[c]	Glycerolipid metabolism	2.3.1.15	'R00851'	[87, 88]
			G3PAT1819Zh	glycerol-3-phosphate acyltransferase ((9Z)-C18:1)	glyc3p[h] + octe9ACP[h] -> 1odec9eg3p[h] + ACP[h]	Glycerolipid metabolism	2.3.1.15	'R00851'	[87, 88]
			G3PAT181h	glycerol-3-phosphate acyltransferase ((11Z)-C18:1)	glyc3p[h] + octeACP[h] -> 1odec11eg3p[h] + ACP[h]	Glycerolipid metabolism	2.3.1.15	'R00851'	[87, 88]
maker_Scaffold_1030-augustus-gene-0.12'	0.008145081	3.993345964	APPTh	ATP:propanoate phosphotransferase, chloroplast	atp[h] + ppa[h] <=> adp[h] + ppap[h]	Propanoate metabolism	2.7.2.1	'R01353'	
			APPTm	ATP:propanoate phosphotransferase, mitochondria	atp[m] + ppa[m] <=> adp[m] + ppap[m]	Propanoate metabolism	2.7.2.1	'R01353'	
			ACKrh	acetate kinase,	ac[h] + atp[h] <=>	Pyruvate	2.7.2.1	'R00315	

				chloroplast	actp[h] + adp[h]	metabolism		'
			ACKrm	acetate kinase, mitochondrial	ac[m] + atp[m] <=> actp[m] + adp[m]	Pyruvate metabolism	2.7.2.1	'R00315'
maker_Scaffold_106-snap-gene-0.69'	0.009245846	4.53302595	SBP	sedoheptulose-bisphosphatase	h2o[h] + s17bp[h] -> pi[h] + s7p[h]	Carbon fixation	3.1.3.37	'R01845'
maker_Scaffold_625-augustus-gene-0.79'	0.010121518	4.962347583	ETHAPT1801819Z	CDP-ethanolamine: diacylglycerol ethanolamine phosphotransferase (18:0/18:1(9Z))	12dgr1801819Z[c] + cdpea[c] -> cmp[c] + h[c] + pe1801819Z[c]	Glycerophospholipid metabolism	2.7.8.1	'R02057' [87, 88, 99]
			ETHAPT18111Z1819Z	CDP-ethanolamine: diacylglycerol ethanolamine phosphotransferase (18:1(11Z)/18:1(9Z))	12dgr18111Z1819Z[c] + cdpea[c] -> cmp[c] + h[c] + pe18111Z1819Z[c]	Glycerophospholipid metabolism	2.7.8.1	'R02057' [87, 88, 99]
			ETHAPT1819Z1819Z	CDP-ethanolamine: diacylglycerol ethanolamine phosphotransferase (18:1(9Z)/18:1(9Z))	12dgr1819Z1819Z[c] + cdpea[c] -> cmp[c] + h[c] + pe1819Z1819Z[c]	Glycerophospholipid metabolism	2.7.8.1	'R02057' [87, 88, 99]
genemark_Scaffold_2835-abinit-gene-0.1'	0.010319731	5.0595272	ASPCT	aspartate carbamoyltransferase, cytosol	asp-L[c] + cbp[c] -> cbasp[c] + pi[c]	Pyrimidine metabolism	2.1.3.2	'R01397'
			ASPCTm	aspartate carbamoyltransferase, mitochondria	asp-L[m] + cbp[m] -> cbasp[m] + pi[m]	Pyrimidine metabolism	2.1.3.2	'R01397'
maker_Scaffold_1851-snap-gene-0.13'	0.015246765	7.475138706	FE3t	ferric iron uptake, plasma membrane	fe3[e] -> fe3[c]	Transport, extracellular	9.A.10.1.6; 9.A.10.1.7	[79]

			FEROt	ferric iron updtake (oxidizing), plasma membrane	4 fe2[e] + 4 h[e] + o2[e] -> 4 fe3[c] + 2 h2o[e]	Transport, extracellular	9.A.10.1.5	[79]
			ASCBOR	L-ascorbate:oxygen oxidoreductase	2 ascb-L[u] + o2[u] -> 2 dhdascb[u] + 2 h2o[u]	Ascorbate and aldarate metabolism	1.10.3.3	'R00068'
			UDPGLCURH	UDP-glucuronate glucuronohydrolase	h2o[c] + udpglcur[c] -> glcur[c] + h[c] + udp[c]	Ascorbate and aldarate metabolism	2.4.1.17	'R08615'
augustus_masked_Scaffold_13-abinit-gene-0.17'	0.015246765	7.475138706	ACSERSULL	O3-Acetyl-L-serine acetate-lyase (adding hydrogen sulfide), cytosol	acser[c] + tsul[c] -> ac[c] + h[c] + scys-L[c]	Cysteine and methionine metabolism	2.5.1.47;2.5.1.48	'R03132'
			ACSERSULLh	O3-Acetyl-L-serine acetate-lyase (adding hydrogen sulfide), chloroplast	acser[h] + tsul[h] -> ac[h] + h[h] + scys-L[h]	Cysteine and methionine metabolism	2.5.1.47;2.5.1.48	'R03132'
			ACSERSULLm	O3-Acetyl-L-serine acetate-lyase (adding hydrogen sulfide), mitochondria	acser[m] + tsul[m] -> ac[m] + h[m] + scys-L[m]	Cysteine and methionine metabolism	2.5.1.47;2.5.1.48	'R03132'
			CYSS	cysteine synthase	acser[h] + h2s[h] -> ac[h] + cys-L[h]	Cysteine metabolism	2.5.1.47	'R00897'
			CYSS(trdrd)	cysteine synthase, thioredoxin	acser[h] + trdrd[h] + tsul[h] -> ac[h] + cys-L[h] + h[h] + hso3[h] + trdox[h]	Cysteine metabolism	2.5.1.47	'R04859'
			DAOTO	2'-Deoxyadenosine 5'-	adp[c] + trdrd[c] ->	Purine	1.17.4.1	'R02017

				diphosphate:oxidized-thioredoxin 2'-oxidoreductase	dadp[c] + h2o[c] + trdox[c]	metabolism		'
			DGOTO	2'-Deoxyguanosine 5'-diphosphate:oxidized-thioredoxin 2'-oxidoreductase	gdp[c] + trdrd[c] -> dgdp[c] + h2o[c] + trdox[c]	Purine metabolism	1.17.4.1	'R02019'
			DCDT	2'-Deoxycytidine diphosphate:oxidized-thioredoxin 2'-oxidoreductase	cdp[c] + trdrd[c] -> dcdp[c] + h2o[c] + trdox[c]	Pyrimidine metabolism	1.17.4.1	'R02024'
			DUDT	2'-Deoxyuridine 5'-diphosphate:oxidized-thioredoxin 2'-oxidoreductase	trdrd[c] + udp[c] -> dudp[c] + h2o[c] + trdox[c]	Pyrimidine metabolism	1.17.4.1	'R02018'
			ACSERL	O3-Acetyl-L-serine acetate-lyase (adding hydrogen sulfide), cytosol	acser[c] + seln[c] <=> ac[c] + 2 h[c] + selcys[c]	Selenoamino acid metabolism	2.5.1.47	'R03601'
			ACSERLh	O3-Acetyl-L-serine acetate-lyase (adding hydrogen sulfide), chloroplast	acser[h] + seln[h] <=> ac[h] + 2 h[h] + selcys[h]	Selenoamino acid metabolism	2.5.1.47	'R03601'
			ACSERLm	O3-Acetyl-L-serine acetate-lyase (adding hydrogen sulfide), mitochondria	acser[m] + seln[m] <=> ac[m] + 2 h[m] + selcys[m]	Selenoamino acid metabolism	2.5.1.47	'R03601'
genemark_Scaffold_70	0.015246765	7.475138706	DAOTO	2'-Deoxyadenosine 5'-	adp[c] + trdrd[c] ->	Purine	1.17.4.1	'R02017

4-abinit-gene-0.31'				diphosphate:oxidized-thioredoxin 2'-oxidoreductase	dadp[c] + h2o[c] + trdox[c]	metabolism	'	
			DGOTO	2'-Deoxyguanosine 5'-diphosphate:oxidized-thioredoxin 2'-oxidoreductase	gdp[c] + trdrd[c] -> dgdp[c] + h2o[c] + trdox[c]	Purine metabolism	1.17.4.1	'R02019'	
			DCDT	2'-Deoxycytidine diphosphate:oxidized-thioredoxin 2'-oxidoreductase	cdp[c] + trdrd[c] -> dcdp[c] + h2o[c] + trdox[c]	Pyrimidine metabolism	1.17.4.1	'R02024'	
			DUDT	2'-Deoxyuridine 5'-diphosphate:oxidized-thioredoxin 2'-oxidoreductase	trdrd[c] + udp[c] -> dudp[c] + h2o[c] + trdox[c]	Pyrimidine metabolism	1.17.4.1	'R02018'	
maker_Scaffold_332-augustus-gene-0.46'	0.015247083	7.475294396	IAGT	inositol 3-alpha-galactosyltransferase	inost[c] + udpgal[c] -> 1Dgali[c] + h[c] + udp[c]	Galactose metabolism	2.4.1.123	'R01192'	[100-104]

APPENDIX C

APPENDIX C 12 potential gene after single gene deletion using LMOMA, Mixotophy

Genes	Knocked-out genes flux value	Mutant/wildtype ratio	Reaction name in iCZ843	Reaction description	Formula	Subsystem	EC Number	KEGG ID	References
genemark_Scaffold_335-abinit-gene-0.8'	0.004986182	1.140335345	MAGAH160	1-acylglycerol acylhydrolase (16:0)	h2o[c] + mag160[c] -> glyc[c] + h[c] + hdca[c]	Glycerolipid metabolism	3.1.1.23	'R01351'	
			MAGAH180	1-acylglycerol acylhydrolase (18:0)	h2o[c] + mag180[c] -> glyc[c] + h[c] + ocdca[c]	Glycerolipid metabolism	3.1.1.23	'R01351'	
			MAGAH18111Z	1-acylglycerol acylhydrolase (18:1(11Z))	h2o[c] + mag18111Z[c] -> glyc[c] + h[c] + ocdcea[c]	Glycerolipid metabolism	3.1.1.23	'R01351'	
			MAGAH1819Z	1-acylglycerol acylhydrolase (18:1(9Z))	h2o[c] + mag1819Z[c] -> glyc[c] + h[c] + ocdce9a[c]	Glycerolipid metabolism	3.1.1.23	'R01351'	
			DHR	dihydroorotase	cbasp[m] <=> dhor-S[m] + h[m] + h2o[m]	Pyrimidine metabolism	3.5.2.3	'R01993'	
maker_Scaffold_1137-augustus-gene-0.114'	0.013857066	3.169098674	TKT1h	transketolase 1	r5p[h] + xu5p-D[h] <=> g3p[h] + s7p[h]	Pentose phosphate pathway;Carbon fixation	2.2.1.1	'R01641'	[93]
			TKT2h	transketolase 2	e4p[h] + xu5p-D[h] <=> f6p-B[h] + g3p[h]	Pentose phosphate pathway;Carbon fixation	2.2.1.1	'R01830'	[93]
genemark_Scaffold_608-abinit-gene-0.22'	0.01625463	3.71741952	ANXANASCOR	antheraxanthin:ascorbate oxidoreductase	anxan[u] + ascb-L[u] <=> dhdascb[u] +	Carotenoid biosynthesis	1.10.99.3	'R07179'	[105]

Genes	Knocked-out genes flux value	Mutant/wildtype ratio	Reaction name in iCZ843	Reaction description	Formula	Subsystem	EC Number	KEGG ID	References
			VIOXANOR	violaxanthin:ascorbate oxidoreductase	h2o[u] + zaxan[u] ascb-L[u] + vioxan[u] <=> anxan[u] + dhdascb[u] + h2o[u]	Carotenoid biosynthesis	1.10.99.3	'R07178'	[105]
maker_Scaffold_85-augustus-gene-0.54'	0.016254881	3.717476863	ADNK	adenosine kinase	adn[c] + atp[c] -> adp[c] + amp[c] + h[c]	Purine metabolism	2.7.1.20	'R00185'	
			ADNKm	adenosine kinase, mitochondria	adn[m] + atp[m] -> adp[m] + amp[m] + h[m]	Purine metabolism	2.7.1.20	'R00185'	
maker_Scaffold_1221-augustus-gene-0.36'	0.016254881	3.717476863	HBCO(nadp)	3-Hydroxybutanoyl-CoA:NADP+ oxidoreductase	aacoa[c] + h[c] + nadph[c] <=> 3hbcoa[c] + nadp[c]	Butanoate metabolism	1.1.1.157	'R01976'	
			HBCO(nadp)m	3-Hydroxybutanoyl-CoA:NADP+ oxidoreductase, mitochondria	aacoa[m] + h[m] + nadph[m] <=> 3hbcoa[m] + nadp[m]	Butanoate metabolism	1.1.1.157	'R01976'	
			HACD2m	3-hydroxyacyl-CoA dehydrogenase (C6:0)	3ohcoa[m] + h[m] + nadh[m] <=> 3hhcoa[m] + nad[m]	Fatty acid elongation in mitochondria	1.1.1.35	'R04748'	[87, 88]
			HACD3m	3-hydroxyacyl-CoA dehydrogenase (C8:0)	3oocoa[m] + h[m] + nadh[m] <=> 3hocoa[m] + nad[m]	Fatty acid elongation in mitochondria	1.1.1.35	'R04745'	[87, 88]
			HACD4m	3-hydroxyacyl-CoA dehydrogenase (C10:0)	3odcoa[m] + h[m] + nadh[m] <=> 3hdcoa[m] + nad[m]	Fatty acid elongation in mitochondria	1.1.1.35	'R04743'	[87, 88]
			HACD5m	3-hydroxyacyl-CoA	3oddcoa[m] + h[m] +	Fatty acid	1.1.1.35	'R04741	[87, 88]

Genes	Knocked-out genes flux value	Mutant/wildtype ratio	Reaction name in iCZ843	Reaction description	Formula	Subsystem	EC Number	KEGG ID	References
				dehydrogenase (C12:0)	nadh[m] <=> 3hddcoa[m] + nad[m]	elongation in mitochondria		'	
			HACD6m	3-hydroxyacyl-CoA dehydrogenase (C14:0)	3otdcoa[m] + h[m] + nadh[m] <=> 3htdcoa[m] + nad[m]	Fatty acid elongation in mitochondria	1.1.1.35	'R04739 '	[87, 88]
			HACD7m	3-hydroxyacyl-CoA dehydrogenase (C16:0)	3ohdcoa[m] + h[m] + nadh[m] <=> 3hhdcoa[m] + nad[m]	Fatty acid elongation in mitochondria	1.1.1.35	'R04737 '	[87, 88]
			ECOAH1	(S)-3-Hydroxybutanoyl-CoA hydro-lyase	b2coa[x] + h2o[x] <=> 3hbcoa[x]	Fatty acid metabolism	4.2.1.17	'R03026 '	
			ECOAH2	(S)-Hydroxyhexanoyl-CoA hydro-lyase	h2o[x] + hx2coa[x] <=> 3hhcoa[x]	Fatty acid metabolism	4.2.1.17	'R04749 '	
			ECOAH3	(S)-Hydroxyoctanoyl-CoA hydro-lyase	h2o[x] + oc2coa[x] <=> 3hocoa[x]	Fatty acid metabolism	4.2.1.17	'R04746 '	
			ECOAH4	(S)-Hydroxydecanoyl-CoA hydro-lyase	dc2coa[x] + h2o[x] <=> 3hdcoa[x]	Fatty acid metabolism	4.2.1.17	'R04744 '	
			ECOAH5	(S)-3-Hydroxydodecanoyl-CoA hydro-lyase	dd2coa[x] + h2o[x] <=> 3hddcoa[x]	Fatty acid metabolism	4.2.1.17	'R04170 '	
			ECOAH6	(S)-3-Hydroxytetradecanoyl-CoA hydro-lyase	h2o[x] + td2coa[x] <=> 3htdcoa[x]	Fatty acid metabolism	4.2.1.17	'R04740 '	
			ECOAH7	(S)-3-Hydroxyhexadecanoyl-CoA hydro-lyase	h2o[x] + hdd2coa[x] <=> 3hhdcoa[x]	Fatty acid metabolism	4.2.1.17	'R04738 '	
			HACD1	(S)-3-Hydroxybutanoyl-	3hbcoa[x] + nad[x]	Fatty acid	1.1.1.35	'R01975	

Genes	Knocked-out genes flux value	Mutant/wildtype ratio	Reaction name in iCZ843	Reaction description	Formula	Subsystem	EC Number	KEGG ID	References
				CoA:NAD oxidoreductase	<=> aacoa[x] + h[x] + nadh[x]	metabolism		'	
			HACD2	(S)-hydroxyhexanoyl-CoA:NAD oxidoreductase	3hhcoa[x] + nad[x] <=> 3ohcoa[x] + h[x] + nadh[x]	Fatty acid metabolism	1.1.1.35	'R04748 '	
			HACD3	(S)-hydroxyoctanoyl-CoA:NAD oxidoreductase	3hocoa[x] + nad[x] <=> 3oocoa[x] + h[x] + nadh[x]	Fatty acid metabolism	1.1.1.35	'R04745 '	
			HACD4	(S)-hydroxydecanoyl-CoA:NAD oxidoreductase	3hdcoa[x] + nad[x] <=> 3odcoa[x] + h[x] + nadh[x]	Fatty acid metabolism	1.1.1.35	'R04743 '	
			HACD5	(S)-3-hydroxydodecanoyl-CoA:NAD oxidoreductase	3hddcoa[x] + nad[x] <=> 3oddcoa[x] + h[x] + nadh[x]	Fatty acid metabolism	1.1.1.35	'R04741 '	
			HACD6	(S)-3-Hydroxytetradecanoyl-CoA:NAD oxidoreductase	3htdcoa[x] + nad[x] <=> 3otdcoa[x] + h[x] + nadh[x]	Fatty acid metabolism	1.1.1.35	'R04739 '	
			HACD7	(S)-3-Hydroxyhexadecanoyl-CoA:NAD oxidoreductase	3hhdcoa[x] + nad[x] <=> 3ohdcoa[x] + h[x] + nadh[x]	Fatty acid metabolism	1.1.1.35	'R04737 '	
maker_Scaffold_308-augustus-gene-0.94'	0.018013395	4.119647483	LCYA	lycopene cyclase (alpha-carotene producing)	dcaro[h] <=> acaro[h]	Carotenoid biosynthesis	1.14.-.-	'R06962 '	[106]
			LCYB	lycopene cyclase (beta-carotene producing)	gcaro[u] <=> caro[u]	Carotenoid biosynthesis	1.14.-.-	'R03824 '	[106]
			LCYG	lycopene cyclase (gamma-carotene	lyc[h] <=> gcaro[h]	Carotenoid biosynthesis	1.14.-.-	'R05341 '	[106]

Genes	Knocked-out genes flux value	Mutant/wildtype ratio	Reaction name in iCZ843	Reaction description	Formula	Subsystem	EC Number	KEGG ID	References
				producing)					
			NEOXANS	neoxanthin synthase	vioxan[u] <=> neoxan[u]	Carotenoid biosynthesis	5.3.99.9	'R06948'	[107]
genemark_Scaffold_1057-abinit-gene-0.17'	0.018013414	4.119651827	LNLCth	linoleic acid transport, chloroplast	lnlc[c] <=> lnlc[h]	Transport, chloroplast	9.B.39.1.1		
			CAROtu	beta carotene transport (Thylakoid Lumen)	caro[u] <=> caro[h]	Transport, thylakoid lumen			[84]
maker_Scaffold_106-snap-gene-0.69'	0.019404977	4.437901059	SBP	sedoheptulose-bisphosphatase	h2o[h] + s17bp[h] -> pi[h] + s7p[h]	Carbon fixation	3.1.3.37	'R01845'	
maker_Scaffold_1801-augustus-gene-0.22'	0.019987454	4.571113008	AACP1819ZS	long-chain-fatty-acid-[acyl-carrier-protein] ligase (18:1(9Z))	ACP[h] + atp[h] + ocdce9a[h] -> amp[h] + h[h] + octe9ACP[h] + ppi[h]	Fatty acid metabolism	6.2.1.20	'R01406'	
			AACPS1	long-chain-fatty-acid-[acyl-carrier-protein] ligase (14:0)	ACP[h] + atp[h] + ttdca[h] -> amp[h] + h[h] + myrsACP[h] + ppi[h]	Fatty acid metabolism	6.2.1.20	'R01406'	
			AACPS3	long-chain-fatty-acid-[acyl-carrier-protein] ligase (16:0)	ACP[h] + atp[h] + hdca[h] -> amp[h] + h[h] + palmACP[h] + ppi[h]	Fatty acid metabolism	6.2.1.20	'R01406'	
			AACPS4	long-chain-fatty-acid-[acyl-carrier-protein] ligase (16:1(9Z))	ACP[h] + atp[h] + hdcea[h] -> amp[h] + h[h] + hdeACP[h] + ppi[h]	Fatty acid metabolism	6.2.1.20	'R01406'	

Genes	Knocked-out genes flux value	Mutant/wildtype ratio	Reaction name in iCZ843	Reaction description	Formula	Subsystem	EC Number	KEGG ID	References
			AACPS5	long-chain-fatty-acid-[acyl-carrier-protein] ligase (18:1(11Z))	ACP[h] + atp[h] + ocdcea[h] -> amp[h] + h[h] + octeACP[h] + ppi[h]	Fatty acid metabolism	6.2.1.20	'R01406'	
			AACPS6	long-chain-fatty-acid-[acyl-carrier-protein] ligase (18:0)	ACP[h] + atp[h] + ocdca[h] -> amp[h] + h[h] + ocdcaACP[h] + ppi[h]	Fatty acid metabolism	6.2.1.20	'R01406'	
			AGPAT1601819Zh	1-hexadecanoyl-sn-glycerol 3-phosphate O-acyltransferase ((9Z)-C18:1) (ACP substrate)	1hdecg3p[h] + octe9ACP[h] -> ACP[h] + pa1601819Z[h]	Glycerolipid metabolism	2.3.1.51	'R02241'	
			AGPAT160h	1-hexadecanoyl-sn-glycerol 3-phosphate O-acyltransferase (n-C16:0) (ACP substrate)	1hdecg3p[h] + palmACP[h] -> ACP[h] + pa160[h]	Glycerolipid metabolism	2.3.1.51	'R02241'	
			AGPAT1801819Zh	1-octadecanoyl-sn-glycerol 3-phosphate O-acyltransferase ((9Z)-C18:1) (ACP substrate)	1odecg3p[h] + octe9ACP[h] -> ACP[h] + pa1801819Z[h]	Glycerolipid metabolism	2.3.1.51	'R02241'	
			AGPAT18111Z160h	1-octadec-11-enoyl-sn-glycerol 3-phosphate O-acyltransferase (n-C16:0) (ACP substrate)	1odec11eg3p[h] + palmACP[h] -> ACP[h] + pa18111Z160[h]	Glycerolipid metabolism	2.3.1.51	'R02241'	
			AGPAT18111Z1819Zh	1-octadec-11-enoyl-sn-glycerol 3-phosphate O-	1odec11eg3p[h] + octe9ACP[h] -> ACP[h]	Glycerolipid metabolism	2.3.1.51	'R02241'	

Genes	Knocked-out genes flux value	Mutant/wildtype ratio	Reaction name in iCZ843	Reaction description	Formula	Subsystem	EC Number	KEGG ID	References
				acyltransferase ((9Z)-C18:1) (ACP substrate)	+ pa18111Z1819Z[h]				
			AGPAT1819Z160h	1-octadec-9-enoyl-sn-glycerol 3-phosphateO-acyltransferase (n-C16:0) (ACP substrate)	1odec9eg3p[h] + palmACP[h] -> ACP[h] + pa1819Z160[h]	Glycerolipid metabolism	2.3.1.51	'R02241'	
			AGPAT1819Z1619Zh	1-octadec-9-enoyl-sn-glycerol 3-phosphateO-acyltransferase ((9Z)-C16:1) (ACP substrate)	1odec9eg3p[h] + hdeACP[h] -> ACP[h] + pa1819Z1619Z[h]	Glycerolipid metabolism	2.3.1.51	'R02241'	
			AGPAT1819Z18111Zh	1-octadec-9-enoyl-sn-glycerol 3-phosphateO-acyltransferase ((11Z)-C18:1) (ACP substrate)	1odec9eg3p[h] + octeACP[h] -> ACP[h] + pa1819Z18111Z[h]	Glycerolipid metabolism	2.3.1.51	'R02241'	
			AGPAT1819Z1819Zh	1-octadec-9-enoyl-sn-glycerol 3-phosphateO-acyltransferase ((9Z)-C18:1) (ACP substrate)	1odec9eg3p[h] + octe9ACP[h] -> ACP[h] + pa1819Z1819Z[h]	Glycerolipid metabolism	2.3.1.51	'R02241'	
			G3PAT160	glycerol-3-phosphate: acyl-coa acyltransferase (16:0)	glyc3p[c] + pmtcoa[c] -> 1hdecg3p[c] + coa[c]	Glycerolipid metabolism	2.3.1.15	'R00851'	[87, 88]
			G3PAT160h	glycerol-3-phosphate acyltransferase (C16:0)	glyc3p[h] + palmACP[h] -> 1hdecg3p[h] + ACP[h]	Glycerolipid metabolism	2.3.1.15	'R00851'	[87, 88]
			G3PAT180	glycerol-3-phosphate: acyl-coa acyltransferase	glyc3p[c] + ocdccoa[c] -> 1odecg3p[c] +	Glycerolipid metabolism	2.3.1.15	'R00851'	[87, 88]

Genes	Knocked-out genes flux value	Mutant/wildtype ratio	Reaction name in iCZ843	Reaction description	Formula	Subsystem	EC Number	KEGG ID	References
				(18:0)	coa[c]				
			G3PAT180h	glycerol-3-phosphate acyltransferase (C18:0)	glyc3p[h] + ocdcaACP[h] -> 1odecg3p[h] + ACP[h]	Glycerolipid metabolism	2.3.1.15	'R00851'	[87, 88]
			G3PAT181	glycerol-3-phosphate: acyl-coa acyltransferase (18:1(11Z))	glyc3p[c] + ocdcecoa[c] -> 1odec11eg3p[c] + coa[c]	Glycerolipid metabolism	2.3.1.15	'R00851'	[87, 88]
			G3PAT1819Z	glycerol-3-phosphate: acyl-coa acyltransferase (18:1(9Z))	glyc3p[c] + ocdce9coa[c] -> 1odec9eg3p[c] + coa[c]	Glycerolipid metabolism	2.3.1.15	'R00851'	[87, 88]
			G3PAT1819Zh	glycerol-3-phosphate acyltransferase ((9Z)-C18:1)	glyc3p[h] + octe9ACP[h] -> 1odec9eg3p[h] + ACP[h]	Glycerolipid metabolism	2.3.1.15	'R00851'	[87, 88]
			G3PAT181h	glycerol-3-phosphate acyltransferase ((11Z)-C18:1)	glyc3p[h] + octeACP[h] -> 1odec11eg3p[h] + ACP[h]	Glycerolipid metabolism	2.3.1.15	'R00851'	[87, 88]
maker_Scaffold_33-augustus-gene-0.119'	0.024301924	5.557828452	MDHx	malate dehydrogenase (NAD)	h[x] + nadh[x] + oaa[x] <=> mal-L[x] + nad[x]	TCA cycle;Carbon fixation;CO2 fixation	1.1.1.37	'R00342'	[81, 82]

APPENDIX D

APPENDIX D Genes data under heterotrophic condition after single-gene deletion screening for top 5 genes assorted by colour

Reaction name	Reaction description	Formula	Genes	Subsystem	EC Number	KEGG ID	References
ETFQO	Electron transfer flavoprotein-ubiquinone oxidoreductase	etfrd[m] + q8[m] -> etfox[m] + q8h2[m]	genemark_Scaffold_1016-abinit-gene-0.16	Cofactor recycling	1.5.5.1	'R04433'	
THRAL	threonine ammonia-lyase (serine)	ser-L[h] <=> nh4[h] + pyr[h]	maker_Scaffold_439-snap-gene-0.203	Glycine, serine and threonine metabolism	4.3.1.19	'R00220'	
TAL	threonine ammonia-lyase	thr-L[h] -> 2obut[h] + nh4[h]	maker_Scaffold_439-snap-gene-0.203	Valine, leucine and isoleucine biosynthesis	4.3.1.19	'R00996'	
GLYDH	Glycerate dehydrogenase	h[c] + hpyr[c] + nadh[c] -> glyc-R[c] + nad[c]	maker_Scaffold_145-augustus-gene-0.53	Glyoxylate metabolism	1.1.1.29	'R01388'	
glycltdhCv	Glycerate dehydrogenase, chloroplast	glx[h] + h[h] + nadh[h] -> glyclt[h] + nad[h]	maker_Scaffold_145-augustus-gene-0.53	Glyoxylate and dicarboxylate metabolism	1.1.1.29	'R00717'	[98]
glydhhCv	Glycerate dehydrogenase, chloroplast	h[h] + nadh[h] + hpyr[h] -> glyc-R[h] + nad[h]	maker_Scaffold_145-augustus-gene-0.53	Glyoxylate and dicarboxylate metabolism	1.1.1.29	'R01388'	[98]
ACOA100OR	Decanoyl-CoA:(acceptor) 2,3-oxidoreductase	dcacoa[x] + fad[x] -> dc2coa[x] + fadh2[x]	genemark_Scaffold_1220-abinit-gene-0.13 genemark_Scaffold_97-abinit-gene-0.20 maker_Scaffold_245-augustus-gene-0.27	Fatty acid metabolism	1.3.99.-;1.3.3.6	'R04754'	

Reaction name	Reaction description	Formula	Genes	Subsystem	EC Number	KEGG ID	References
			maker_Scaffold_58-augustus-gene-0.54				
ACOA120OR	Lauroyl-CoA:(acceptor) 2,3-oxidoreductase	ddcacoa[x] + fad[x] -> dd2coa[x] + fadh2[x]	genemark_Scaffold_1220-abinit-gene-0.13 genemark_Scaffold_97-abinit-gene-0.20 maker_Scaffold_245-augustus-gene-0.27 maker_Scaffold_58-augustus-gene-0.54	Fatty acid metabolism	1.3.99.-;1.3.3.6	'R03857'	
ACOA140OR	Tetradecanoyl-CoA:(acceptor) 2,3-oxidoreductase	fad[x] + tdcoa[x] -> fadh2[x] + td2coa[x]	genemark_Scaffold_1220-abinit-gene-0.13 genemark_Scaffold_97-abinit-gene-0.20 maker_Scaffold_245-augustus-gene-0.27 maker_Scaffold_58-augustus-gene-0.54	Fatty acid metabolism	1.3.99.-;1.3.3.6	'R03990'	
ACOA160OR	Palmitoyl-CoA:oxygen 2-oxidoreductase	fad[x] + pmtcoa[x] -> fadh2[x] + hdd2coa[x]	genemark_Scaffold_1220-abinit-gene-0.13 genemark_Scaffold_97-abinit-gene-0.20 maker_Scaffold_245-augustus-gene-0.27 maker_Scaffold_58-augustus-gene-0.54	Fatty acid metabolism	1.3.99.-;1.3.3.6	'R01279'	
ACOA40OR	Butanoyl-CoA:oxygen 2-oxidoreductase	btcoa[x] + fad[x] -> b2coa[x] + fadh2[x]	genemark_Scaffold_1220-abinit-gene-0.13 genemark_Scaffold_97-abinit-gene-0.20 maker_Scaffold_245-augustus-gene-0.27 maker_Scaffold_58-augustus-gene-0.54	Fatty acid metabolism	1.3.99.-;1.3.99.2;1.3.3.6	'R01175'	
ACOA60OR	Hexanoyl-CoA:(acceptor) 2,3-oxidoreductase	fad[x] + hxcoa[x] -> fadh2[x] + hx2coa[x]	genemark_Scaffold_1220-abinit-gene-0.13 genemark_Scaffold_97-abinit-gene-0.20 maker_Scaffold_245-augustus-gene-0.27 maker_Scaffold_58-augustus-gene-0.54	Fatty acid metabolism	1.3.99.-;1.3.99.2;1.3.3.6	'R04751'	
ACOA80OR	Octanoyl-CoA:oxygen 2-oxidoreductase	fad[x] + occoa[x] -> fadh2[x] + oc2coa[x]	genemark_Scaffold_1220-abinit-gene-0.13 genemark_Scaffold_97-abinit-gene-0.20 maker_Scaffold_245-augustus-gene-0.27 maker_Scaffold_58-augustus-gene-0.54	Fatty acid metabolism	1.3.99.-;1.3.3.6	'R03777'	
PCNO	propanoyl-CoA:NADP+ 2-	nadp[c] + ppcoa[c] <=> h[c] + nadph[c] +	genemark_Scaffold_1220-abinit-gene-0.13	Propanoate	1.3.1.-	'R00919	

Reaction name	Reaction description	Formula	Genes	Subsystem	EC Number	KEGG ID	References
	oxidoreductase	prpncoa[c]		metabolism		'	
OPC8COAOR	OPC-8:0 CoA:oxygen 2-oxidoreductase	fad[c] + opc8coa[c] -> fadh2[c] + opc8coat2e[c]	genemark_Scaffold_1220-abinit-gene-0.13 genemark_Scaffold_2362-abinit-gene-0.3 genemark_Scaffold_97-abinit-gene-0.20 maker_Scaffold_149-augustus-gene-0.129 maker_Scaffold_245-augustus-gene-0.27 maker_Scaffold_58-augustus-gene-0.54	alpha-Linolenic acid metabolism	1.3.3.6	'R07888'	[96, 97]
OPC6COAOR	OPC-6:0 CoA:oxygen 2-oxidoreductase	fad[c] + opc6coa[c] -> fadh2[c] + opc6coat2e[c]	genemark_Scaffold_1220-abinit-gene-0.13 genemark_Scaffold_2362-abinit-gene-0.3 genemark_Scaffold_97-abinit-gene-0.20 maker_Scaffold_149-augustus-gene-0.129 maker_Scaffold_245-augustus-gene-0.27 maker_Scaffold_58-augustus-gene-0.54	alpha-Linolenic acid metabolism	1.3.3.6	'R07892'	[96, 97]
OPC4COAOR	OPC-4:0 CoA:oxygen 2-oxidoreductase	fad[c] + opc4coa[c] -> fadh2[c] + opc4coat2e[c]	genemark_Scaffold_1220-abinit-gene-0.13 genemark_Scaffold_2362-abinit-gene-0.3 genemark_Scaffold_97-abinit-gene-0.20 maker_Scaffold_149-augustus-gene-0.129 maker_Scaffold_245-augustus-gene-0.27 maker_Scaffold_58-augustus-gene-0.54	alpha-Linolenic acid metabolism	1.3.3.6	'R07896'	[96, 97]
STARCH300DEGR2A	degradation of starch300 by phosphorylase, amylase, dextrinase, maltase (chloroplast), 1:5 glc-A:g1p (dark-dep rxn)	49 h2o[h] + 250 pi[h] + starch300[h] -> 250 g1p[h] + 50 glc-A[h]	augustus_masked_Scaffold_1605-abinit-gene-0.4 maker_Scaffold_1099-snap-gene-0.7 maker_Scaffold_1153-augustus-gene-0.48 maker_Scaffold_120-snap-gene-0.43 maker_Scaffold_240-snap-gene-0.258 maker_Scaffold_678-snap-gene-0.98	Starch metabolism	2.4.1.1 + 3.2.1.1 + 3.2.1.2 + 3.2.1.142 + 3.2.1.20	'R02111 + R02108 + R02112 + R00028'	[94, 95]

Reaction name	Reaction description	Formula	Genes	Subsystem	EC Number	KEGG ID	References
STARCH300 DEGR2B	degradation of starch300 by phosphorylase, amylase, dextrinase, maltase (chloroplast), 1:5 glc-B:g1p (dark-dep rxn)	49 h2o[h] + 250 pi[h] + starch300[h] -> 250 g1p[h] + 50 glc-B[h]	augustus_masked_Scaffold_1605-abinit-gene-0.4 maker_Scaffold_1099-snap-gene-0.7 maker_Scaffold_1153-augustus-gene-0.48 maker_Scaffold_120-snap-gene-0.43 maker_Scaffold_240-snap-gene-0.258 maker_Scaffold_678-snap-gene-0.98	Starch metabolism	2.4.1.1 + 3.2.1.1 + 3.2.1.2 + 3.2.1.142 + 3.2.1.20	'R02111 + R02108 + R02112 + R00028'	[94, 95]
STARCH300 DEGRA	degradation of starch300 by phosphorylase, amylase, dextrinase, maltase (chloroplast), 1:3 glc-A:g1p (light-dep rxn)	74 h2o[h] + 225 pi[h] + starch300[h] -> 225 g1p[h] + 75 glc-A[h]	augustus_masked_Scaffold_1605-abinit-gene-0.4 maker_Scaffold_1099-snap-gene-0.7 maker_Scaffold_1153-augustus-gene-0.48 maker_Scaffold_120-snap-gene-0.43 maker_Scaffold_240-snap-gene-0.258 maker_Scaffold_678-snap-gene-0.98	Starch metabolism	2.4.1.1 + 3.2.1.1 + 3.2.1.2 + 3.2.1.142 + 3.2.1.20	'R02111 + R02108 + R02112 + R00028'	[94, 95]
STARCH300 DEGRB	degradation of starch300 by phosphorylase, amylase, dextrinase, maltase (chloroplast), 1:3 glc-B:g1p (light-dep rxn)	74 h2o[h] + 225 pi[h] + starch300[h] -> 225 g1p[h] + 75 glc-B[h]	augustus_masked_Scaffold_1605-abinit-gene-0.4 maker_Scaffold_1099-snap-gene-0.7 maker_Scaffold_1153-augustus-gene-0.48 maker_Scaffold_120-snap-gene-0.43 maker_Scaffold_240-snap-gene-0.258 maker_Scaffold_678-snap-gene-0.98	Starch metabolism	2.4.1.1 + 3.2.1.1 + 3.2.1.2 + 3.2.1.142 + 3.2.1.20	'R02111 + R02108 + R02112 + R00028'	[94, 95]

APPENDIX E

APPENDIX E Genes data under photoautotrophic condition after single-gene deletion screening for top 5 genes assorted by colour

Reaction name	Reaction description	Formula	Genes	Subsystem	EC Number	KEGG ID	References
IAGT	inositol 3-alpha-galactosyltransferase	inost[c] + udpgal[c] -> 1Dgali[c] + h[c] + udp[c]	maker_Scaffold_332-augustus-gene-0.46	Galactose metabolism	2.4.1.123	'R01192'	[100-104]
DUDT	2'-Deoxyadenosine 5'-diphosphate:oxidized-thioredoxin 2'-oxidoreductase	adp[c] + trdrd[c] -> dadp[c] + h2o[c] + trdox[c]	augustus_masked_Scaffold_13-abinit-gene-0.17 augustus_masked_Scaffold_89-abinit-gene-1.30 genemark_Scaffold_24-abinit-gene-0.26 genemark_Scaffold_704-abinit-gene-0.31	Purine metabolism	1.17.4.1	'R02017'	
DCDT	2'-Deoxyguanosine 5'-diphosphate:oxidized-thioredoxin 2'-oxidoreductase	gdp[c] + trdrd[c] -> dgdp[c] + h2o[c] + trdox[c]	augustus_masked_Scaffold_13-abinit-gene-0.17 augustus_masked_Scaffold_89-abinit-gene-1.30 genemark_Scaffold_24-abinit-gene-0.26 genemark_Scaffold_704-abinit-gene-0.31	Purine metabolism	1.17.4.1	'R02019'	
DGOTO	2'-Deoxycytidine diphosphate:oxidized-thioredoxin 2'-oxidoreductase	cdp[c] + trdrd[c] -> dcdp[c] + h2o[c] + trdox[c]	augustus_masked_Scaffold_13-abinit-gene-0.17 augustus_masked_Scaffold_89-abinit-gene-1.30 genemark_Scaffold_24-abinit-gene-0.26 genemark_Scaffold_704-abinit-gene-0.31	Pyrimidine metabolism	1.17.4.1	'R02024'	
DAOTO	2'-Deoxyuridine 5'-diphosphate:oxidized-thioredoxin 2'-oxidoreductase	trdrd[c] + udp[c] -> dudp[c] + h2o[c] + trdox[c]	augustus_masked_Scaffold_13-abinit-gene-0.17 augustus_masked_Scaffold_89-abinit-gene-1.30 genemark_Scaffold_24-abinit-gene-0.26 genemark_Scaffold_704-abinit-gene-0.31	Pyrimidine metabolism	1.17.4.1	'R02018'	
ACSERLm	O3-Acetyl-L-serine acetate-lyase	acser[c] + tsul[c] -> ac[c] + h[c] + scys-L[c]	augustus_masked_Scaffold_13-abinit-gene-	Cysteine and	2.5.1.47;2.	'R03132	

Reaction name	Reaction description	Formula	Genes	Subsystem	EC Number	KEGG ID	References
	(adding hydrogen sulfide), cytosol		0.17 maker_Scaffold_1404-augustus-gene-0.24 maker_Scaffold_1860-snap-gene-0.39 maker_Scaffold_338-augustus-gene-0.67 maker_Scaffold_732-augustus-gene-0.46 maker_Scaffold_86-augustus-gene-0.117	methionine metabolism	5.1.48	'	
ACSERLh	O3-Acetyl-L-serine acetate-lyase (adding hydrogen sulfide), chloroplast	acser[h] + tsul[h] -> ac[h] + h[h] + scys-L[h]	augustus_masked_Scaffold_13-abinit-gene-0.17 maker_Scaffold_1404-augustus-gene-0.24 maker_Scaffold_1860-snap-gene-0.39 maker_Scaffold_338-augustus-gene-0.67 maker_Scaffold_86-augustus-gene-0.117	Cysteine and methionine metabolism	2.5.1.47;2.5.1.48	'R03132'	
ACSERL	O3-Acetyl-L-serine acetate-lyase (adding hydrogen sulfide), mitochondria	acser[m] + tsul[m] -> ac[m] + h[m] + scys-L[m]	augustus_masked_Scaffold_13-abinit-gene-0.17 maker_Scaffold_1404-augustus-gene-0.24 maker_Scaffold_338-augustus-gene-0.67 maker_Scaffold_86-augustus-gene-0.117	Cysteine and methionine metabolism	2.5.1.47;2.5.1.48	'R03132'	
DUDT	cysteine synthase	acser[h] + h2s[h] -> ac[h] + cys-L[h]	augustus_masked_Scaffold_13-abinit-gene-0.17 maker_Scaffold_1404-augustus-gene-0.24 maker_Scaffold_338-augustus-gene-0.67 maker_Scaffold_732-augustus-gene-0.46 maker_Scaffold_86-augustus-gene-0.117	Cysteine metabolism	2.5.1.47	'R00897'	
DCDT	cysteine synthase, thioredoxin	acser[h] + trdrd[h] + tsul[h] -> ac[h] + cys-L[h] + h[h] + hso3[h] + trdox[h]	augustus_masked_Scaffold_13-abinit-gene-0.17 augustus_masked_Scaffold_797-abinit-gene-0.1 genemark_Scaffold_242-abinit-gene-0.5 genemark_Scaffold_875-abinit-gene-0.68 maker_Scaffold_1404-	Cysteine metabolism	2.5.1.47	'R04859'	

Reaction name	Reaction description	Formula	Genes	Subsystem	EC Number	KEGG ID	References
			augustus-gene-0.24 maker_Scaffold_1792-snap-gene-0.41 maker_Scaffold_324-augustus-gene-0.51 maker_Scaffold_338-augustus-gene-0.67 maker_Scaffold_732-augustus-gene-0.46 maker_Scaffold_86-augustus-gene-0.117				
DGOTO	2'-Deoxyadenosine 5'-diphosphate:oxidized-thioredoxin 2'-oxidoreductase	adp[c] + trdrd[c] -> dadp[c] + h2o[c] + trdox[c]	augustus_masked_Scaffold_13-abinit-gene-0.17 augustus_masked_Scaffold_89-abinit-gene-1.30 genemark_Scaffold_24-abinit-gene-0.26 genemark_Scaffold_704-abinit-gene-0.31	Purine metabolism	1.17.4.1	'R02017'	
DAOTO	2'-Deoxyguanosine 5'-diphosphate:oxidized-thioredoxin 2'-oxidoreductase	gdp[c] + trdrd[c] -> dgdp[c] + h2o[c] + trdox[c]	augustus_masked_Scaffold_13-abinit-gene-0.17 augustus_masked_Scaffold_89-abinit-gene-1.30 genemark_Scaffold_24-abinit-gene-0.26 genemark_Scaffold_704-abinit-gene-0.31	Purine metabolism	1.17.4.1	'R02019'	
CYSS(trdrd)	2'-Deoxycytidine diphosphate:oxidized-thioredoxin 2'-oxidoreductase	cdp[c] + trdrd[c] -> dcdp[c] + h2o[c] + trdox[c]	augustus_masked_Scaffold_13-abinit-gene-0.17 augustus_masked_Scaffold_89-abinit-gene-1.30 genemark_Scaffold_24-abinit-gene-0.26 genemark_Scaffold_704-abinit-gene-0.31	Pyrimidine metabolism	1.17.4.1	'R02024'	
CYSS	2'-Deoxyuridine 5'-diphosphate:oxidized-thioredoxin 2'-oxidoreductase	trdrd[c] + udp[c] -> dudp[c] + h2o[c] + trdox[c]	augustus_masked_Scaffold_13-abinit-gene-0.17 augustus_masked_Scaffold_89-abinit-gene-1.30 genemark_Scaffold_24-abinit-gene-0.26 genemark_Scaffold_704-abinit-gene-0.31	Pyrimidine metabolism	1.17.4.1	'R02018'	

Reaction name	Reaction description	Formula	Genes	Subsystem	EC Number	KEGG ID	References
ACSERSULLm	O3-Acetyl-L-serine acetate-lyase (adding hydrogen sulfide), cytosol	acser[c] + seln[c] <=> ac[c] + 2 h[c] + selcys[c]	augustus_masked_Scaffold_13-abinit-gene-0.17 maker_Scaffold_1404-augustus-gene-0.24 maker_Scaffold_338-augustus-gene-0.67 maker_Scaffold_732-augustus-gene-0.46 maker_Scaffold_86-augustus-gene-0.117	Selenoamino acid metabolism	2.5.1.47	'R03601'	
ACSERSULLh	O3-Acetyl-L-serine acetate-lyase (adding hydrogen sulfide), chloroplast	acser[h] + seln[h] <=> ac[h] + 2 h[h] + selcys[h]	augustus_masked_Scaffold_13-abinit-gene-0.17 maker_Scaffold_1404-augustus-gene-0.24 maker_Scaffold_338-augustus-gene-0.67 maker_Scaffold_86-augustus-gene-0.117	Selenoamino acid metabolism	2.5.1.47	'R03601'	
ACSERSULL	O3-Acetyl-L-serine acetate-lyase (adding hydrogen sulfide), mitochondria	acser[m] + seln[m] <=> ac[m] + 2 h[m] + selcys[m]	augustus_masked_Scaffold_13-abinit-gene-0.17 maker_Scaffold_1404-augustus-gene-0.24 maker_Scaffold_338-augustus-gene-0.67 maker_Scaffold_86-augustus-gene-0.117	Selenoamino acid metabolism	2.5.1.47	'R03601'	
UDPGLCURH	ferric iron uptake, plasma membrane	fe3[e] -> fe3[c]	maker_Scaffold_1851-snap-gene-0.13	Transport, extracellular	9.A.10.1.6; 9.A.10.1.7		[79]
ASCBOR	ferric iron updtake (oxidizing), plasma membrane	4 fe2[e] + 4 h[e] + o2[e] -> 4 fe3[c] + 2 h2o[e]	maker_Scaffold_1851-snap-gene-0.13	Transport, extracellular	9.A.10.1.5		[79]
FEROt	L-ascorbate:oxygen oxidoreductase	2 ascb-L[u] + o2[u] -> 2 dhdascb[u] + 2 h2o[u]	maker_Scaffold_1851-snap-gene-0.13	Ascorbate and aldarate metabolism	1.10.3.3	'R00068'	
FE3t	UDP-glucuronate glucuronohydrolase	h2o[c] + udpglcur[c] -> glcur[c] + h[c] + udp[c]	maker_Scaffold_1851-snap-gene-0.13	Ascorbate and aldarate metabolism	2.4.1.17	'R08615'	

Reaction name	Reaction description	Formula	Genes	Subsystem	EC Number	KEGG ID	References
ASPCTm	aspartate carbamoyltransferase, cytosol	asp-L[c] + cbp[c] -> cbasp[c] + pi[c]	genemark_Scaffold_2835-abinit-gene-0.1	Pyrimidine metabolism	2.1.3.2	'R01397'	
ASPCT	aspartate carbamoyltransferase, mitochondria	asp-L[m] + cbp[m] -> cbasp[m] + pi[m]	genemark_Scaffold_2835-abinit-gene-0.1	Pyrimidine metabolism	2.1.3.2	'R01397'	

APPENDIX F

APPENDIX F Genes data under mixotrophic condition after single-gene deletion screening for top 5 genes assorted by colour

Reaction name	Reaction description	Formula	Genes	Subsystem	EC Number	KEGG ID	References
MDHx	malate dehydrogenase (NAD)	h[x] + nadh[x] + oaa[x] <=> mal-L[x] + nad[x]	maker_Scaffold_33-augustus-gene-0.119	TCA cycle;Carbon fixation;CO2 fixation	1.1.1.37	'R00342'	[81, 82]
G3PAT181h	long-chain-fatty-acid-[acyl-carrier-protein] ligase (18:1(9Z))	ACP[h] + atp[h] + ocdce9a[h] -> amp[h] + h[h] + octe9ACP[h] + ppi[h]	genemark_Scaffold_2156-abinit-gene-0.5 maker_Scaffold_1801-augustus-gene-0.22	Glycerolipid metabolism	6.2.1.20	'R00851'	
G3PAT1819Zh	long-chain-fatty-acid-[acyl-carrier-protein] ligase (14:0)	ACP[h] + atp[h] + ttdca[h] -> amp[h] + h[h] + myrsACP[h] + ppi[h]	genemark_Scaffold_2156-abinit-gene-0.5 maker_Scaffold_1801-augustus-gene-0.22	Glycerolipid metabolism	6.2.1.20	'R00851'	
G3PAT1819Z	long-chain-fatty-acid-[acyl-carrier-protein] ligase (16:0)	ACP[h] + atp[h] + hdca[h] -> amp[h] + h[h] + palmACP[h] + ppi[h]	genemark_Scaffold_2156-abinit-gene-0.5 maker_Scaffold_1801-augustus-gene-0.22	Glycerolipid metabolism	6.2.1.20	'R00851'	
G3PAT181	long-chain-fatty-acid-[acyl-carrier-protein] ligase (16:1(9Z))	ACP[h] + atp[h] + hdcea[h] -> amp[h] + h[h] + hdeACP[h] + ppi[h]	genemark_Scaffold_2156-abinit-gene-0.5 maker_Scaffold_1801-augustus-gene-0.22	Glycerolipid metabolism	6.2.1.20	'R00851'	
G3PAT180h	long-chain-fatty-acid-[acyl-carrier-protein] ligase (18:1(11Z))	ACP[h] + atp[h] + ocdcea[h] -> amp[h] + h[h] + octeACP[h] + ppi[h]	genemark_Scaffold_2156-abinit-gene-0.5 maker_Scaffold_1801-augustus-gene-0.22	Glycerolipid metabolism	6.2.1.20	'R00851'	
G3PAT180	long-chain-fatty-acid-[acyl-carrier-protein] ligase (18:0)	ACP[h] + atp[h] + ocdca[h] -> amp[h] + h[h] + ocdcaACP[h] + ppi[h]	genemark_Scaffold_2156-abinit-gene-0.5 maker_Scaffold_1801-augustus-gene-0.22	Glycerolipid metabolism	6.2.1.20	'R00851'	
G3PAT160h	1-hexadecanoyl-sn-glycerol 3-phosphate O-acyltransferase ((9Z)-C18:1) (ACP substrate)	1hdecg3p[h] + octe9ACP[h] -> ACP[h] + pa1601819Z[h]	maker_Scaffold_1801-augustus-gene-0.22 maker_Scaffold_3256-snap-gene-0.9	Glycerolipid metabolism	2.3.1.51	'R00851'	
G3PAT160	1-hexadecanoyl-sn-glycerol 3-phosphate O-acyltransferase (n-C16:0) (ACP substrate)	1hdecg3p[h] + palmACP[h] -> ACP[h] + pa160[h]	maker_Scaffold_1801-augustus-gene-0.22 maker_Scaffold_3256-snap-gene-0.9	Glycerolipid metabolism	2.3.1.51	'R00851'	
AGPAT1819	1-octadecanoyl-sn-glycerol 3-	1odecg3p[h] + octe9ACP[h] -> ACP[h] +	maker_Scaffold_1801-augustus-gene-0.22	Glycerolipid	2.3.1.51	'R02241'	

Reaction name	Reaction description	Formula	Genes	Subsystem	EC Number	KEGG ID	References
Z1819Zh	phosphate O-acyltransferase ((9Z)-C18:1) (ACP substrate)	pa1801819Z[h]	maker_Scaffold_3256-snap-gene-0.9	metabolism			
AGPAT1819Z18111Zh	1-octadec-11-enoyl-sn-glycerol 3-phosphate O-acyltransferase (n-C16:0) (ACP substrate)	1odec11eg3p[h] + palmACP[h] -> ACP[h] + pa18111Z160[h]	maker_Scaffold_1801-augustus-gene-0.22 maker_Scaffold_3256-snap-gene-0.9	Glycerolipid metabolism	2.3.1.51	'R02241'	
AGPAT1819Z1619Zh	1-octadec-11-enoyl-sn-glycerol 3-phosphate O-acyltransferase ((9Z)-C18:1) (ACP substrate)	1odec11eg3p[h] + octe9ACP[h] -> ACP[h] + pa18111Z1819Z[h]	maker_Scaffold_1801-augustus-gene-0.22 maker_Scaffold_3256-snap-gene-0.9	Glycerolipid metabolism	2.3.1.51	'R02241'	
AGPAT1819Z160h	1-octadec-9-enoyl-sn-glycerol 3-phosphateO-acyltransferase (n-C16:0) (ACP substrate)	1odec9eg3p[h] + palmACP[h] -> ACP[h] + pa1819Z160[h]	maker_Scaffold_1801-augustus-gene-0.22 maker_Scaffold_3256-snap-gene-0.9	Glycerolipid metabolism	2.3.1.51	'R02241'	
AGPAT18111Z1819Zh	1-octadec-9-enoyl-sn-glycerol 3-phosphateO-acyltransferase ((9Z)-C16:1) (ACP substrate)	1odec9eg3p[h] + hdeACP[h] -> ACP[h] + pa1819Z1619Z[h]	maker_Scaffold_1801-augustus-gene-0.22 maker_Scaffold_3256-snap-gene-0.9	Glycerolipid metabolism	2.3.1.51	'R02241'	
AGPAT18111Z160h	1-octadec-9-enoyl-sn-glycerol 3-phosphateO-acyltransferase ((11Z)-C18:1) (ACP substrate)	1odec9eg3p[h] + octeACP[h] -> ACP[h] + pa1819Z18111Z[h]	maker_Scaffold_1801-augustus-gene-0.22 maker_Scaffold_3256-snap-gene-0.9	Glycerolipid metabolism	2.3.1.51	'R02241'	
AGPAT1801819Zh	1-octadec-9-enoyl-sn-glycerol 3-phosphateO-acyltransferase ((9Z)-C18:1) (ACP substrate)	1odec9eg3p[h] + octe9ACP[h] -> ACP[h] + pa1819Z1819Z[h]	maker_Scaffold_1801-augustus-gene-0.22 maker_Scaffold_3256-snap-gene-0.9	Glycerolipid metabolism	2.3.1.51	'R02241'	
AGPAT160h	glycerol-3-phosphate: acyl-coa acyltransferase (16:0)	glyc3p[c] + pmtcoa[c] -> 1hdecg3p[c] + coa[c]	maker_Scaffold_1801-augustus-gene-0.22	Glycerolipid metabolism	2.3.1.15	'R02241'	[87, 88]
AGPAT1601819Zh	glycerol-3-phosphate acyltransferase (C16:0)	glyc3p[h] + palmACP[h] -> 1hdecg3p[h] + ACP[h]	maker_Scaffold_1801-augustus-gene-0.22	Glycerolipid metabolism	2.3.1.15	'R02241'	[87, 88]
AACPS6	glycerol-3-phosphate: acyl-coa acyltransferase (18:0)	glyc3p[c] + ocdccoa[c] -> 1odecg3p[c] + coa[c]	maker_Scaffold_1801-augustus-gene-0.22	Fatty acid metabolism	2.3.1.15	'R01406'	[87, 88]

Reaction name	Reaction description	Formula	Genes	Subsystem	EC Number	KEGG ID	References
AACPS5	glycerol-3-phosphate acyltransferase (C18:0)	glyc3p[h] + ocdcaACP[h] -> 1odecg3p[h] + ACP[h]	maker_Scaffold_1801-augustus-gene-0.22	Fatty acid metabolism	2.3.1.15	'R01406'	[87, 88]
AACPS4	glycerol-3-phosphate: acyl-coa acyltransferase (18:1(11Z))	glyc3p[c] + ocdcecoa[c] -> 1odec11eg3p[c] + coa[c]	maker_Scaffold_1801-augustus-gene-0.22	Fatty acid metabolism	2.3.1.15	'R01406'	[87, 88]
AACPS3	glycerol-3-phosphate: acyl-coa acyltransferase (18:1(9Z))	glyc3p[c] + ocdce9coa[c] -> 1odec9eg3p[c] + coa[c]	maker_Scaffold_1801-augustus-gene-0.22	Fatty acid metabolism	2.3.1.15	'R01406'	[87, 88]
AACPS1	glycerol-3-phosphate acyltransferase ((9Z)-C18:1)	glyc3p[h] + octe9ACP[h] -> 1odec9eg3p[h] + ACP[h]	maker_Scaffold_1801-augustus-gene-0.22	Fatty acid metabolism	2.3.1.15	'R01406'	[87, 88]
AACP1819ZS	glycerol-3-phosphate acyltransferase ((11Z)-C18:1)	glyc3p[h] + octeACP[h] -> 1odec11eg3p[h] + ACP[h]	maker_Scaffold_1801-augustus-gene-0.22	Fatty acid metabolism	2.3.1.15	'R01406'	[87, 88]
SBP	sedoheptulose-bisphosphatase	h2o[h] + s17bp[h] -> pi[h] + s7p[h]	maker_Scaffold_106-snap-gene-0.69	Carbon fixation	3.1.3.37	'R01845'	
CAROtu	linoleic acid transport, chloroplast	lnlc[c] <=> lnlc[h]	genemark_Scaffold_1057-abinit-gene-0.17	Transport, chloroplast	9.B.39.1.1		[84]
LNLCth	beta carotene transport (Thylakoid Lumen)	caro[u] <=> caro[h]	genemark_Scaffold_1057-abinit-gene-0.17	Transport, thylakoid lumen			[84]
NEOXANS	lycopene cyclase (alpha-carotene producing)	dcaro[h] <=> acaro[h]	maker_Scaffold_308-augustus-gene-0.94	Carotenoid biosynthesis	1.14.-.-	'R06948'	[106]
LCYG	lycopene cyclase (beta-carotene producing)	gcaro[u] <=> caro[u]	maker_Scaffold_308-augustus-gene-0.94	Carotenoid biosynthesis	1.14.-.-	'R05341'	[106]
LCYB	lycopene cyclase (gamma-carotene producing)	lyc[h] <=> gcaro[h]	maker_Scaffold_308-augustus-gene-0.94	Carotenoid biosynthesis	1.14.-.-	'R03824'	[106]
LCYA	neoxanthin synthase	vioxan[u] <=> neoxan[u]	maker_Scaffold_143-augustus-gene-0.218 maker_Scaffold_308-augustus-gene-0.94	Carotenoid biosynthesis	5.3.99.9	'R06962'	[107]

APPENDIX G

APPENDIX G The results of BLASTX of desirable gene found in heterotrophic condition.

Gene	Reaction description in UTEX 395	BLASTX	Organism	E-value	Percent Identity	Accesion number
'genemark_Scaffold_10	Electron transfer flavoprotein-	FMN-linked oxidoreductases superfamily				
16-abinit-gene-0.16'	ubiquinone oxidoreductase	protein	Raphanus sativus	1E-30	0.4375	KAJ4874827.1
		FMN-linked oxidoreductases superfamily				
		protein	Arabidopsis thaliana	9E-29	0.3947	KAF5184181.1
maker_Scaffold_439-						
snap-gene-0.203'	threonine ammonia-lyase (serine)	threonine dehydratase	*Monoraphidium neglectum*	5.00E-38	76.64%	XP_013898811.1
	threonine ammonia-lyase	threonine deaminase isoform A	*Chlorella sorokiniana*	6.00E-24	59.46%	PRW39107.1
		threonine dehydratase	*Monoraphidium neglectum*	1.00E-16	42.45%	XP_013900919.1
maker_Scaffold_145-						
augustus-gene-0.53'	Glycerate dehydrogenase	glycerate dehydrogenase	*Micractinium conductrix*	7.00E-38	84.27%	PSC68951.1
	Glycerate dehydrogenase, chloroplast	putative Glycerate dehydrogenase HPR	*Chlorella desiccata*	1.00E-34	76.40%	KAH7615606.1
	Glycerate dehydrogenase, chloroplast	glycerate dehydrogenase	*Chlorella sorokiniana*	2.00E-34	82.02%	PRW60721.1
		glycerate dehydrogenase HPR	*Selaginella moellendorffii*	8.00E-29	70.79%	XP_024536185.1
		glycerate dehydrogenase HPR	*Zingiber officinale*	4.00E-26	67.42%	XP_042434957.1
genemark_Scaffold_122	Decanoyl-CoA:(acceptor) 2,3-					
0-abinit-gene-0.13'	oxidoreductase	Acyl-coenzyme A oxidase peroxisomal	*Chlorella sorokiniana*	1.00E-30	80.00%	PRW59738.1
	Lauroyl-CoA:(acceptor) 2,3-					
	oxidoreductase	Acyl-coenzyme A oxidase peroxisomal	*Micractinium conductrix*	7.00E-30	85.00%	PSC71354.1
	Tetradecanoyl-CoA:(acceptor) 2,3-					
	oxidoreductase	acyl-coenzyme A oxidase peroxisomal-like	*Trebouxia sp. A1-2*	5.00E-29	73.42%	KAA6417273.1
	Palmitoyl-CoA:oxygen 2-oxidoreductase	Acyl-coenzyme A oxidase 4	*Pleodorina starrii*	1.00E-27	78.75%	GLC46413.1
	Butanoyl-CoA:oxygen 2-oxidoreductase	Acyl-coenzyme A oxidase 4, peroxisomal	*Tetrabaena socialis*	4.00E-27	71.25%	PNH03628.1

Gene	Reaction description in UTEX 395	BLASTX	Organism	E-value	Percent Identity	Accesion number
	Hexanoyl-CoA:(acceptor) 2,3-oxidoreductase Octanoyl-CoA:oxygen 2-oxidoreductase propanoyl-CoA:NADP+ 2-oxidoreductase OPC-8:0 CoA:oxygen 2-oxidoreductase OPC-6:0 CoA:oxygen 2-oxidoreductase OPC-4:0 CoA:oxygen 2-oxidoreductase					
'maker_Scaffold_1153-augustus-gene-0.48'	degradation of starch300 by phosphorylase, amylase, dextrinase, maltase (chloroplast), 1:5 glc-A:g1p (dark-dep rxn)	Beta-amylase chloroplastic isoform A	*Chlorella sorokiniana*	7.00E-15	73.85%	PRW56984.1
	degradation of starch300 by phosphorylase, amylase, dextrinase, maltase (chloroplast), 1:5 glc-B:g1p (dark-dep rxn)	Beta-amylase chloroplastic	*Micractinium conductrix*	3.00E-14	72.31%	PSC72195.1
	degradation of starch300 by phosphorylase, amylase, dextrinase, maltase (chloroplast), 1:3 glc-A:g1p (light-dep rxn)	putative Beta-amylase 2, chloroplastic	*Chlorella desiccata*	7.00E-11	68.33%	KAH7622131.1
	degradation of starch300 by phosphorylase, amylase, dextrinase, maltase (chloroplast), 1:3 glc-B:g1p (light-dep rxn)	beta-amylase-2	*Coccomyxa sp.*	1.00E-05	61.22%	BBJ25298.1
		beta-amylase	*Dunaliella salina*	1.00E-05	59.18%	KAF5827053.1

APPENDIX H

APPENDIX H The results of BLASTX of desirable gene found in photoautotrophic condition.

Gene	Reaction description in UTEX 395	BLASTX	Organism	E-value	Percent Identity	Accesion number
maker_Scaffold_332-augustus-gene-0.46'	inositol 3-alpha-galactosyltransferase	inositol phosphorylceramide glucuronosyltransferase 1	Phalaenopsis equestris	1.00E-15	48.81%	XP_020570779.1
		inositol phosphorylceramide glucuronosyltransferase 1 isoform X2	Brassica napus	3.00E-15	47.13%	XP_048602397.1
		inositol phosphorylceramide glucuronosyltransferase 1	Eutrema salsugineum	4.00E-15	48.28%	XP_006400396.1
		inositol phosphorylceramide glucuronosyltransferase 1	Capsella rubella	4.00E-15	48.28%	XP_006287478.1
		inositol phosphorylceramide glucuronosyltransferase 1	Brassica napus	4.00E-15	48.28%	XP_013681872.2
'genemark_Scaffold_704-abinit-gene-0.31'	2'-Deoxyadenosine 5'-diphosphate:oxidized-thioredoxin 2'-oxidoreductase	ribonucleoside-diphosphate reductase small chain	Micractinium conductrix	1.00E-51	93.14%	PSC67582.1
	2'-Deoxyguanosine 5'-diphosphate:oxidized-thioredoxin 2'-oxidoreductase	hypothetical protein COHA_009814	Chlorella ohadii	2.00E-50	87.50%	KAI7836306.1
	2'-Deoxycytidine diphosphate:oxidized-thioredoxin 2'-oxidoreductase	ribonucleoside-diphosphate reductase small chain	Chlorella sorokiniana	3.00E-49	90.20%	PRW39307.1
	2'-Deoxyuridine 5'-diphosphate:oxidized-thioredoxin 2'-oxidoreductase	Putative ribonucleoside-diphosphate reductase small chain B	Auxenochlorella protothecoides	3.00E-43	78.43%	XP_011397294.1
		putative ribonucleoside-diphosphate reductase small chain	Scenedesmus sp. NREL 46B-D3	1.00E-42	74.51%	KAF6258921.1

Gene	Reaction description in UTEX 395	BLASTX	Organism	E-value	Percent Identity	Accesion number
'augustus_masked_Scaffold_13-abinit-gene-0.17'	O3-Acetyl-L-serine acetate-lyase (adding hydrogen sulfide), cytosol	NADPH-protochlorophyllide oxidoreductase	Haematococcus lacustris	4.00E-31	82.67%	GFH23526.1
	O3-Acetyl-L-serine acetate-lyase (adding hydrogen sulfide), chloroplast	protochlorophyllide oxidoreductase B	Chorispora bungeana	4.00E-27	72.84%	ACJ12925.1
	O3-Acetyl-L-serine acetate-lyase (adding hydrogen sulfide), mitochondria	chloroplast NADPH-protochlorophyllide oxidoreductase	Cucumis sativus	1.00E-26	67.90%	ABK55691.1
	cysteine synthase	protochlorophyllide oxidoreductase A	Arabidopsis thaliana	1.00E-26	71.60%	NP_200230.1
	cysteine synthase, thioredoxin	NADPH:protochlorophyllide oxidoreductase A	Arabidopsis thaliana	1.00E-26	71.60%	AAC49043.1
	2'-Deoxyadenosine 5'-diphosphate:oxidized-thioredoxin 2'-oxidoreductase					
	2'-Deoxyguanosine 5'-diphosphate:oxidized-thioredoxin 2'-oxidoreductase					
	2'-Deoxycytidine diphosphate:oxidized-thioredoxin 2'-oxidoreductase					
	2'-Deoxyuridine 5'-diphosphate:oxidized-thioredoxin 2'-oxidoreductase					
	O3-Acetyl-L-serine acetate-lyase (adding hydrogen sulfide), cytosol					
	O3-Acetyl-L-serine acetate-lyase (adding hydrogen sulfide), chloroplast					
	O3-Acetyl-L-serine acetate-lyase (adding					

Gene	Reaction description in UTEX 395	BLASTX	Organism	E-value	Percent Identity	Accesion number
	hydrogen sulfide), mitochondria					
maker_Scaffold_1851-snap-gene-0.13'	ferric iron uptake, plasma membrane	Copper-transporting ATPase	Actinidia chinensis var. chinensis	3.00E-66	59.18%	PSS14536.1
	ferric iron updtake (oxidizing), plasma membrane	probable copper-transporting ATPase HMA5 isoform X1	Juglans regia	8.00E-66	64.95%	XP_018828654.2
	L-ascorbate:oxygen oxidoreductase	probable copper-transporting ATPase HMA5 isoform X3	Carya illinoinensis	6.00E-65	64.29%	XP_042953084.1
	UDP-glucuronate glucuronohydrolase	copper-transporting ATPase HMA4-like	Quercus suber	1.00E-64	58.16%	XP_023907384.1
		copper-transporting ATPase HMA4-like isoform X1	Cicer arietinum	2.00E-64	61.86%	XP_012574029.1
genemark_Scaffold_2835-abinit-gene-0.1'	aspartate carbamoyltransferase, cytosol	Aspartate carbamoyltransferase chloroplastic	Micractinium conductrix	2.00E-47	84.17%	PSC67787.1
	aspartate carbamoyltransferase, mitochondria	Aspartate carbamoyltransferase chloroplastic	Chlorella sorokiniana	2.00E-45	71.13%	PRW45053.1
		Aspartate/ornithine carbamoyltransferase	Monoraphidium minutum	2.00E-32	53.33%	KAI8472341.1
		Aspartate carbamoyltransferase 2, chloroplastic	Auxenochlorella protothecoides	6.00E-30	71.70%	XP_011401082.1
		Aspartate carbamoyltransferase, chloroplastic	Tetrabaena socialis	2.00E-23	66.33%	PNH06181.1

APPENDIX I

APPENDIX I The results of BLASTX of desirable gene found in mixotrophic condition.

Gene	Reaction description in UTEX 395	BLASTX	Organism	E-value	Percent Identity	Accesion number
maker_Scaffold_1801-augustus-gene-0.22'	long-chain-fatty-acid-[acyl-carrier-protein] ligase (18:1(9Z))	Transmembrane 9 superfamily member 3	*Coccomyxa sp.*	3.00E-24	69.62%	BDA40971.1
	long-chain-fatty-acid-[acyl-carrier-protein] ligase (14:0)	transmembrane 9 superfamily member 2-like	*Phoenix dactylifera*	1.00E-17	55.70%	XP_008813153.2
	long-chain-fatty-acid-[acyl-carrier-protein] ligase (16:0)	transmembrane 9 superfamily	*Klebsormidium nitens*	1.00E-18	59.49%	GAQ83119.1
	long-chain-fatty-acid-[acyl-carrier-protein] ligase (16:1(9Z))	Transmembrane 9 superfamily member 2	*Cocos nucifera*	7.00E-18	53.09%	KAG1360594.1
	long-chain-fatty-acid-[acyl-carrier-protein] ligase (18:1(11Z))	transmembrane 9 superfamily member 2-like	*Asparagus officinalis*	6.00E-33	85.96%	XP_020273471.1
	long-chain-fatty-acid-[acyl-carrier-protein] ligase (18:0)					
	1-hexadecanoyl-sn-glycerol 3-phosphate O-acyltransferase ((9Z)-C18:1) (ACP substrate)					
	1-hexadecanoyl-sn-glycerol 3-phosphate O-acyltransferase (n-C16:0) (ACP substrate)					
	1-octadecanoyl-sn-glycerol 3-					

phosphate O-acyltransferase ((9Z)-C18:1) (ACP substrate)

1-octadec-11-enoyl-sn-glycerol 3-phosphate O-acyltransferase (n-C16:0) (ACP substrate)

1-octadec-11-enoyl-sn-glycerol 3-phosphate O-acyltransferase ((9Z)-C18:1) (ACP substrate)

1-octadec-9-enoyl-sn-glycerol 3-phosphateO-acyltransferase (n-C16:0) (ACP substrate)

1-octadec-9-enoyl-sn-glycerol 3-phosphateO-acyltransferase ((9Z)-C16:1) (ACP substrate)

1-octadec-9-enoyl-sn-glycerol 3-phosphateO-acyltransferase ((11Z)-C18:1) (ACP substrate)

1-octadec-9-enoyl-sn-glycerol 3-phosphateO-acyltransferase ((9Z)-C18:1) (ACP substrate)

glycerol-3-phosphate: acyl-coa acyltransferase (16:0)

glycerol-3-phosphate acyltransferase (C16:0)

glycerol-3-phosphate: acyl-coa acyltransferase (18:0)

glycerol-3-phosphate acyltransferase (C18:0)

	glycerol-3-phosphate: acyl-coa acyltransferase (18:1(11Z)) glycerol-3-phosphate: acyl-coa acyltransferase (18:1(9Z)) glycerol-3-phosphate acyltransferase ((9Z)-C18:1) glycerol-3-phosphate acyltransferase ((11Z)-C18:1) malate dehydrogenase (NAD)					
'maker_Scaffold_106-snap-gene-0.69'	sedoheptulose-bisphosphatase	sedoheptulose-1,7-bisphosphatase	*Chloropicon primus*	1.00E-37	92.86%	QDZ22268.1
		Sedoheptulose-1,7-bisphosphatase, chloroplastic	*Coccomyxa sp. Obi*	1.00E-37	90.48%	BDA47815.1
		Sedoheptulose-1,7-chloroplastic	*Chlorella sorokiniana*	2.00E-37	92.86%	PRW56262.1
		sedoheptulose-1,7-bisphosphatase	*Coccomyxa subellipsoidea*	3.00E-36	86.90%	XP_005642742.1
		sedoheptulose-1,7-bisphosphatase	*Micractinium conductrix*	1.00E-35	91.67%	PSC69040.1
genemark_Scaffold_1057-abinit-gene-0.17'	linoleic acid transport, chloroplast beta carotene transport (Thylakoid Lumen)	hypothetical protein D9Q98_003261	*Chlorella vulgaris*	1.00E-123	99.66%	KAI3433447.1
		hypothetical protein D9Q98_003261	*Chlorella vulgaris*	2.00E-71	93.75%	KAI3433447.1
'maker_Scaffold_308-augustus-gene-0.94'	lycopene cyclase (alpha-carotene producing)	Lycopene beta chloroplastic	*Micractinium conductrix*	7.00E-50	82.03%	PSC67972.1
	lycopene cyclase (beta-carotene producing)	chloroplast lycopene beta cyclase precursor	*Chromochloris zofingiensis*	6.00E-41	70.16%	CBH31263.1
	lycopene cyclase (gamma-carotene	Lycopene beta cyclase,	*Coccomyxa sp. Obi*	2.00E-40	66.40%	BDA46459.1

	producing)	chloroplastic				
	neoxanthin synthase	chloroplast lycopene beta cyclase precursor	*Chlorella sorokiniana*	1.00E-39	83.64%	PRW59266.1
		Lycopene beta cyclase, chloroplastic	*Auxenochlorella protothecoides*	2.00E-38	69.53%	XP_011397795.1
maker_Scaffold_1221-augustus-gene-0.36'	3-Hydroxybutanoyl-CoA:NADP+ oxidoreductase	3-hydroxybutyryl-dehydrogenase	*Chlorella sorokiniana*	8.00E-26	43.41%	PRW33847.1
	3-Hydroxybutanoyl-CoA:NADP+ oxidoreductase, mitochondria	3-hydroxybutyryl-CoA dehydrogenase	*Myxococcales bacterium*	8.00E-25	40.56%	MBR57353.1
	3-hydroxyacyl-CoA dehydrogenase (C6:0)	3-hydroxybutyryl-CoA dehydrogenase	*Pseudogulbenkiania sp. MAI-1*	7.00E-20	53.27%	WP_024302708.1
	3-hydroxyacyl-CoA dehydrogenase (C8:0)	3-hydroxybutyryl-CoA dehydrogenase	*Deltaproteobacteria bacterium*	8.00E-20	62.50%	MBK6689950.1
	3-hydroxyacyl-CoA dehydrogenase (C10:0)	3-hydroxybutyryl-dehydrogenase	*Micractinium conductrix*	3.00E-22	76.06%	PSC70272.1
	3-hydroxyacyl-CoA dehydrogenase (C12:0)					
	3-hydroxyacyl-CoA dehydrogenase (C14:0)					
	3-hydroxyacyl-CoA dehydrogenase (C16:0)					
	(S)-3-Hydroxybutanoyl-CoA hydro-lyase					
	(S)-Hydroxyhexanoyl-CoA hydro-lyase					
	(S)-Hydroxyoctanoyl-CoA hydro-lyase					
	(S)-Hydroxydecanoyl-CoA hydro-lyase					
	(S)-3-Hydroxydodecanoyl-CoA hydro-lyase					

(S)-3-Hydroxytetradecanoyl-CoA hydro-lyase

(S)-3-Hydroxyhexadecanoyl-CoA hydro-lyase

(S)-3-Hydroxybutanoyl-CoA:NAD oxidoreductase

(S)-hydroxyhexanoyl-CoA:NAD oxidoreductase

(S)-hydroxyoctanoyl-CoA:NAD oxidoreductase

(S)-hydroxydecanoyl-CoA:NAD oxidoreductase

(S)-3-hydroxydodecanoyl-CoA:NAD oxidoreductase

(S)-3-Hydroxytetradecanoyl-CoA:NAD oxidoreductase

(S)-3-Hydroxyhexadecanoyl-CoA:NAD oxidoreductase

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