# Drop-In Biofuel production by using fatty acid photodecarboxylase from *Chlorella variabilis* in the oleaginous yeast *Yarrowia lipolytica*.

[Stefan Bruder][1]

[Eva Johanna Moldenhauer][1]

[Rodrigo Ledesma-Amaro][2]

[Johannes Kabisch] [1]

[1][Computer-aided Synthetic Biology, Technische Universität Darmstadt, Darmstadt, Germany]

[2][Department of Bioengineering and Imperial College Centre for Synthetic Biology, Imperial College London, London, United Kingdom]

**Correspondence:** [Johannes Kabisch, Computer-aided Synthetic Biology, Technische Universität Darmstadt, Schnittspahnstr. 12, 64287 Darmstadt, Germany].

**E-mail**: [kabisch@bio.tu-darmstadt.de]

**Keywords:** [Drop-in biofuels, fatty acid photodecarboxylase, hydrocarbons, alkane, alkene, photoenzyme, oleaginous yeast, *Yarrowia lipolytica*]

**Abbreviations:** [**AAR**, acyl-ACP reductase; **ADO**, aldehyde deformylating oxygenase; ***Cv/r***, *Chlorella variabilis/reinhardtii*; **FFA**, free fatty acids; **GMC,** glucose-methanol-choline; **SCO**, single cell oil; **FAP**, fatty acid photodecarboxylase; **FFA**, free fatty acids; **YaliTAR**, Transformation-associated recombination assisted by *Y. lipolytica*]

**Abstract**

Oleaginous yeasts are potent hosts for the renewable production of lipids, fatty acids and derived products, such as biofuels. Several promising processes have been described that produce hydrocarbon drop-in biofuels based on fatty acid decarboxylation and fatty aldehyde decarbonylation. Unfortunately, besides fatty aldehyde toxicity and high reactivity, the most investigated enzyme, aldehyde-deformylating oxygenase, shows unfavorable catalytic properties which hindered high yields in previous metabolic engineering approaches. To demonstrate an alternative alkane production pathway for oleaginous yeasts, we describe the production of biodiesel-like odd-chain alkanes and alkenes, by heterologously expressing a recently discovered light-driven oxidase from *Chlorella variabilis* (CvFAP) in *Yarrowia lipolytica.* Providing glucose and light in a synthetic medium we achieved titers of 10.87 mg/L of hydrocarbons using a fatty acid overproducing strain.

**Short abstract:**

Oleaginous yeasts are potent hosts for the renewable production of lipids, fatty acids and derived products such as biofuels. Here, we describe, the production of odd-numbered alkanes and alkenes with a length of 17 and 15 carbons by expression a fatty acid photodecarboxylase (CvFAP) from *Chlorella variabilis* in different *Yarrowia lipolytica* strains under blue light exposure.

**Introduction**

Modern human society is built upon readily available hydrocarbons, currently predominantly derived from fossil resources. The depletion of these as well as the adverse effects of their intense utilization have led to a variety of global challenges (Junne and Kabisch, 2017). A concept to counteract these, is to shift towards a bioeconomy by finding novel and drop-in

alternatives produced on the basis of renewable resources. One such alternative are so called drop-in biofuels which are substantially similar to current fuels and are not associated with some of the drawbacks of first generation biofuels like ethanol or fatty acid methyl esters (Junne and Kabisch, 2017).

In recent years a variety of enzymes for the microbial production of hydrocarbons have been discovered and exploited. The most prominently among these is the pair formed by the acyl-ACP reductase (AAR) and the decarbonylating aldehyde-deformylating oxygenase (ADO) discovered in hydrocarbon producing cyanobacteria and expressed in *E. coli* by Schirmer *et al.*, 2010. Following this first proof of concept, the route from fatty acids to hydrocarbons was optimized and transferred to SCO (single cell oil) accumulating organisms.

Oleaginous yeasts are arbitrarily defined as being able to accumulate more than 20% of their dry weight as lipids. Among these, the yeast *Yarrowia lipolytica* is well exploited in respect to genetic amenability and frequently used for industrial applications (Barth, 2013).

The ability to produce large amounts of lipids makes it an attractive host for fatty acid-derived biofuels. Thus, the above-described pathways for hydrocarbon formation have been adapted to *Y. lipolytica* by Xu *et al.*, 2016. Fig. 1A summarizes different strategies for fatty acid-derived hydrocarbon formation with yeasts. A more recent publication identified a promiscuous activity of an algal photoenzyme (Sorigué *et al.*, 2017). This glucose-methanol-choline (GMC) oxidoreductase termed fatty acid photodecarboxylase (FAP) was both found in *Chlorella variabilis* (CvFAP) and *Chlorella reinhardtii* (CrFAP).

In this work, we present the expression of CvFAP for the first time in an oleaginous yeast - *Yarrowia lipolytica*. Using different strain backgrounds, we detected up to 10.87 mg/L of hydrocarbon formation in a strain with an increased pool of free fatty acids (FFA) (Ledesma-Amaro *et al.*, 2016).

**Results and Discussion**

Due to low turnover number, the need of a coupled electron transfer system and the reactive and toxic substrate fatty aldehyde, the most prominently used ADO hindered high hydrocarbon yields in previous metabolic engineering approaches (Tab.1) (Andre *et al.*, 2013; Zhang *et al.*, 2013; Xu *et al.*, 2017). In contrast, the CvFAP enzyme directly utilizes FFA as its substrate as well as the readily available cofactor FAD. The catalysis is directly driven by the photons of blue light and hence tightly controllable. Unlike the AAR/ADO pathway, no additional genes for cofactor recycling are required (Sorigué *et al.*, 2017).

**[Expression and characterization of CvFAP in *Yarrowia lipolytica* using YaliTAR]**

With regard to a rapid characterization, an *in vivo* DNA assembly strategy mediated by *Y. lipolytica* was carried out. In contrast to *S. cerevisiae*, which mainly employs homologous recombination as a DNA repair mechanism, in *Y. lipolytica* non-homologous end-joining (NHEJ) is preferred (Kretzschmar *et al.*, 2013). As a consequence, several DNA assembly methods developed for baker's yeast are not directly transferable or applicable. In previous studies, efficient homologous recombination for genomic integration with short length flanking fragments was successfully shown for *Yarrowia Δku70* mutant strains (Verbeke *et al.*, 2013). In order to transfer the frequently used baker's yeast transformation-associated recombination (TAR) (Kunes *et al.*, 1985) for assembling the centromeric CvFAP expression plasmid (Supp. Fig. S1) within *Yarrowia*, co-transformation of the backbone and corresponding insert in a *Δku70*-strain background was successfully performed.

Positive constructs were grown on lipid-body-inducible YSM medium, containing 5% D-glucose as carbon source, under exposure of a common plant LED-light for 96 h. Under blue light conditions a concentration of 112.1+/-31.4 µg/L of hydrocarbons (alkane/alkenes) could be achieved. In a darkened experimental setup 1.5+/-1 µg/L were produced. The empty vector control revealed no detectable production of hydrocarbons (Suppl., Tab. S1).

**[Alkane production with CvFAP in different *Y. lipolytica* strain backgrounds]**

To evaluate the influence of different strain backgrounds with respect to fatty acid availability, we transformed (protocol by Xuan *et al.*, 1988) the CvFAP expression vector into two different strains (Xuan *et al.*, 1988). We chose the laboratory strain H222 with knocked out beta-oxidation for increased lipid accumulation and deleted *ALK1* gene for inhibition of alkane degradation (S33001), as well as strain JMY5749, an overproducer of FFA, described in (Ledesma-Amaro *et al.*, 2016), for enhanced substrate availability. A blue light LED strip with a more distinct wavelength range was used to provide photons for the light-capturing cofactor. The duration of cultivation was 96 h to impede complete depletion of glucose in order to avoid conceivable alkane degradation (Hirakawa *et al.*, 2009). The cell dry weights of both strains at the end of the cultivation were in a similar range (Suppl., Tab S2). A nearly 30 times higher hydrocarbon concentration was achieved for JMY5749 in opposite to S33001 background under exposure of blue light (Genotype of JMY5749/CvFAP highlighted in Fig. 1B, results are shown in supplementary Fig. S4). The differences in hydrocarbon titers indicate a high potential for further optimizations regarding substrate concentration and prevention of alkane degradation.

Upon an optimisation of extraction and GC analysis (described in Suppl.), a total hydrocarbon concentrations of 10.87+/-1.11 mg/L could be detected (shown in Fig. 2). The intracellular concentrations of detectable odd-numbered alkanes/alkenes correspond to the *Y. lipolytica* fatty acid composition after decarboxylation (In comparison to Suppl., Fig S5). Most of the alkanes produced were heptadecane, 8-heptadecene and 6,9-heptadecadiene in similar levels followed by pentadecane and 7-pentadecene. Furthermore, the intracellular total fatty acids concentration revealed a significant lower amount compared to the empty vector control (Suppl., Fig S5). The hydrocarbon composition also matches the findings of Sorigué *et al*. (2017) and Huijbers *et al.* (2018). The empty vector construct did not show any hydrocarbon formation. No hydrocarbons (alkanes C8-C20) could be detected in the supernatants of any samples (data not shown).

## Concluding remarks

Expression of CvFAP in oleaginous, fatty acid secreting *Yarrowia lipolytica* under blue light exposure leads to the production of odd-numbered alkanes and alkenes with a length of 17 and 15 carbons. Especially the absence of reliable and readily available inducible promoters for *Y. lipolytica* makes this light-driven reaction desirable with respect to process control. For example by omitting the inducing wavelength, the enzyme can be produced from a constitutive promoter and catalysis can be switched on only after sufficient amounts of fatty acids have accumulated. Due to the release of $CO_2$ during decarboxylation, a large production system may have a negative ecological impact. However, recently described carbon dioxide ($CO_2$) fixation approaches could be implemented in further studies (Guadalupe-Medina *et al.*, 2013; Li *et al.*, 2017).

## Authors' Contribution

SB conceived the experimental plan and coordinated the work and analyses. SB and EJM conducted the experimental work. SB, JK and EJM prepared the manuscript. RLA provided the strain JMY5749, revised the manuscript and helped with fruitful discussions. All authors read and approved the final manuscript.

## Acknowledgement

SB and EJM are funded by an BMEL/FNR grant (FKZ: 22007413). RLA received financial support from Imperial College London in the form of a Imperial College Research Fellowship. JK is funded by the CompuGene LOEWE grant.

We gratefully acknowledge Dr. Mislav Oreb for strain H222 *Δku70* and Dr. Alexander Rapp for the provision of the broad range photometer. Thanks to Dr. Thomas Hofmeyer for the

construction of H222 *Δalk1* strain, Ina Menyes for GC-MS measurements and Belinda Escher for general support.

**Conflict of interest**

The authors have declared no conflicts of interest.

**References**

Andre, C., Kim, S.W., Yu, X.-H., and Shanklin, J. (2013) Fusing catalase to an alkane-producing enzyme maintains enzymatic activity by converting the inhibitory byproduct $H_2O_2$ to the cosubstrate $O_2$. *Proc. Natl. Acad. Sci. U. S. A.* **110**: 3191–3196.

Barth, G. ed. (2013) *Yarrowia lipolytica* Springer Berlin Heidelberg, Berlin, Heidelberg.

Cao, Y.-X., Xiao, W.-H., Zhang, J.-L., Xie, Z.-X., Ding, M.-Z., Yuan, Y.-J. (2016) Heterologous biosynthesis and manipulation of alkanes in *Escherichia coli. Metab. Eng.* **38**: 19-28.

Chen, B., Lee, D.-Y., and Chang, M.W. (2015) Combinatorial metabolic engineering of *Saccharomyces cerevisiae* for terminal alkene production. *Metab. Eng.* **31**: 53–61.

Guadalupe-Medina, V., Wisselink, H.W., Luttik, M.A.H., de Hulster, E., Daran, J.-M., Pronk, J.T., van Maris, A.J.A. (2013) Carbon dioxide fixation by Calvin-Cycle enzymes improves ethanol yield in yeast. *Biotechnol. Biofuels* **6**:125.

Hirakawa, K., Kobayashi, S., Inoue, T., Endoh-Yamagami, S., Fukuda, R., and Ohta, A. (2009) Yas3p, an Opi1 family transcription factor, regulates cytochrome P450 expression in response to n-alkanes in *Yarrowia lipolytica*. *J. Biol. Chem.* **284**: 7126–7137.

Huijbers, M.M.E., Zhang, W., Tonin, F., Hollmann, F. (2018) Light-Driven Enzymatic Decarboxylation of Fatty Acids. *Angew. Chem. Int. Ed.* **57**: 13648-13651.

Junne, S. and Kabisch, J. (2017) Fueling the future with biomass: Processes and pathways for a sustainable supply of hydrocarbon fuels and biogas. *Eng. Life Sci.* **17**: 14–26.

Kang, M.-K. and Nielsen, J. (2017) Biobased production of alkanes and alkenes through metabolic engineering of microorganisms. *J. Ind. Microbiol. Biotechnol.* **44**: 613–622.

Kang, M.-K., Zhou, Y.J., Buijs, N.A., and Nielsen, J. (2017) Functional screening of aldehyde decarbonylases for long-chain alkane production by *Saccharomyces cerevisiae*. *Microb. Cell Fact.* **16**: 74.

Kretzschmar, A., Otto, C., Holz, M., Werner, S., Hübner, L., and Barth, G. (2013) Increased homologous integration frequency in Y*arrowia lipolytica* strains defective in non-homologous end-joining. *Curr. Genet.* **59**: 63–72.

Kunes, S., Botstein, D., and Fox, M.S. (1985) Transformation of yeast with linearized plasmid DNA. *J. Mol. Biol.* **184**: 375–387.

Ledesma-Amaro, R., Dulermo, R., Niehus, X., and Nicaud, J.-M. (2016) Combining metabolic engineering and process optimization to improve production and secretion of fatty acids. *Metab. Eng.* **38**: 38–46.

Li, Y.-J., Wang, M.-M., Chen, Y.-W., Wang, M., Fan, L.-H., Tan, T.-W. (2017) Engineered yeast with a $CO_2$-fixation pathway to improve the bio-ethanol production from xylose-mixed sugars. *Sci. Rep.* **7**: 43875.

Matthäus, F., Ketelhot, M., Gatter, M., and Barth, G. (2014) Production of lycopene in the non-carotenoid-producing yeast *Yarrowia lipolytica*. *Appl. Environ. Microbiol.* **80**: 1660–1669.

Papanikolaou, S. and Aggelis, G. (2003) Modeling lipid accumulation and degradation in *Yarrowia lipolytica* cultivated on industrial fats. *Curr. Microbiol.* **46**: 398–402.

Schirmer, A., Rude, M.A., Li, X., Popova, E., and del Cardayre, S.B. (2010) Microbial biosynthesis of alkanes. *Science* **329**: 559–562.

Sorigué, D., Légeret, B., Cuiné, S., Blangy, S., Moulin, S., Billon, E., *et al.* (2017) An algal photoenzyme converts fatty acids to hydrocarbons. *Science* **357**: 903–907.

Verbeke, J., Beopoulos, A., and Nicaud, J.-M. (2013) Efficient homologous recombination with short length flanking fragments in Ku70 deficient *Yarrowia lipolytica s*trains. *Biotechnol. Lett.* **35**: 571–576.

Xuan, J.-W., Fournier, P., and Gaillardin, C. (1988) Cloning of the LYS5 gene encoding saccharopine dehydrogenase from the yeast *Yarrowia lipolytica* by target integration. *Curr. Genet.* **14**: 15–21.

Xu, P., Qiao, K., Ahn, W.S., and Stephanopoulos, G. (2016) Engineering *Yarrowia lipolytica* as a platform for synthesis of drop-in transportation fuels and oleochemicals. *Proc. Natl. Acad. Sci. U. S. A.* **113**: 10848–10853.

Xu, P., Qiao, K., and Stephanopoulos, G. (2017) Engineering oxidative stress defense pathways to build a robust lipid production platform in *Yarrowia lipolytica*: Engineering robust microbial oil cell factory. *Biotechnol. Bioeng.* **114**: 1521–1530.

Zhang, J., Lu, X., and Li, J.-J. (2013) Conversion of fatty aldehydes into alk (a/e)nes by in vitro reconstituted cyanobacterial aldehyde-deformylating oxygenase with the cognate electron transfer system. *Biotechnol. Biofuels* **6**: 86.

Zhou, Y.J., Buijs, N.A., Zhu, Z., Gómez, D.O., Boonsombuti, A., Siewers, V., and Nielsen, J. (2016) Harnessing yeast peroxisomes for biosynthesis of fatty-acid-derived biofuels and chemicals with relieved side-pathway competition. *J. Am. Chem. Soc.* **138:** 15368–15377.

Zhou, Y.J., Hu, Y., Zhu, Z., Siewers, V., and Nielsen, J. (2018) Engineering 1-Alkene biosynthesis and secretion by dynamic regulation in yeast. *ACS Synth. Biol.* **7**: 584–590.

**Table 1.** [Hydrocarbons produced by selected organisms, expressing different heterologous pathways.]

| **Pathway** | **Host** | **Titer** | **Reference** |
|---|---|---|---|
| ARR/ADO | *E.coli* | 1.31 g/L | (Cao *et al.*, 2016) |
| ARR/ADO | *E. coli* | ~ 300 mg/L | (Schirmer *et al.*, 2010) |
| CAR/ADO | *Y. lipolytica* | 23.3 mg/L | (Xu *et al.*, 2016) |

| | | | |
|---|---|---|---|
| CAR/ADO | *S. cerevisiae* | 1.1 mg/L | (Kang *et al.*, 2017) |
| OleT | *S. cerevisiae* | 3.7 mg/L | (Chen *et al.*, 2015) |
| UndB | *S. cerevisiae* | 35.3 mg/L | (Zhou *et al.*, 2018b) |
| CvFAP | *E. coli* | n.d. | (Sorigué *et al*., 2017) |
| CvFAP | *Y. lipolytica* | 10.67 mg/L | This study |

**Table 2.** [*Yarrowia lipolytica* strains used in this study]

| **Strain name** | **Genotype** | **Description** | **Reference** |
|---|---|---|---|
| H222 SW1 | H222 *MATA ura3::suc2 Δku70* | Improved homologous recombination capabilities | Kretzschmar *et al.*, 2013 |
| S33001 | H222 *Δpox1-6 Δalk1::mTFP ura-* | Knocked out beta-oxidation for increased lipid accumulation and deleted ALK1 gene for inhibition of alkane degradation. | AG Kabisch, common strain collection |
| JMY5749 | W29 *Δmfe1 Δfaa1 pTEF_DGA2 pTEF_TGL4 pTEF_KlTGL3* | FFA overproducing strain. referred as JMY5479 in (Ledesma-Amaro *et al.*, 2016) | |

**Table 3.** [*Yarrowia* mineral Salt Medium (YSM) for the induction of lipid droplet (LD) formation based on Papanikolaou and Aggelis, 2003 and Matthäus *et al.*, 2014. The medium was composed as a cost-effective alternative to common LD inducible media and designed for fed-batch cultivations with *Y. lipolytica*].

| **Base compounds** | **Chemical formula** | **Conc. [g/L]** |
|---|---|---|
| Sodium phosphate dibasic | $Na_2HPO_4 \cdot 2H_2O$ | 1.6 |
| Potassium dihydrogen phosphate | $KH_2PO4$ | 0.092 |
| Ammonium sulfate | $(NH_4)_2SO_4$ | 0.5 |

| | | |
|---|---|---|
| Further supplements | | |
| Magnesium sulfate heptahydrate | $MgSO_4 \cdot 7H_2O$ | 0.7 |
| Calcium chloride hexahydrate | $CaCl_2 \cdot 6H_2O$ | 0.1 |
| yeast extract | - | 0.5 |
| D-glucose | - | 50 |
| Trace elements | | |
| Boric acid | $H_3BO_3$ | 0.0005 |
| Manganese sulfate monohydrate | $MnSO_4 \cdot H_2O$ | 0.0004 |
| Zinc sulfate heptahydrate | $ZnSO_4 \cdot 7H_2O$ | 0.0004 |
| Ferric chloride hexahydrate | $FeCl_3 \cdot 6H_2O$ | 0.006 |

**Figure legends**

**Figure 1.** [A: Previously described pathways for hydrocarbon production with yeast (modified from (Xu *et al.*, 2016)). *Y. lipolytica* enzymes are shown in green, intracellular metabolites in black. Orange coloured enzymes are investigated in (Xu *et al.*, 2016), dark blue coloured enzymes are reviewed in (Kang and Nielsen, 2017). B: Expression of CvFAP, with modifications of strain JMY5749 are shown in red, characterized in this study.]

**Figure 2**. [Alkane production with CvFAP expressed in *Yarrowia* JMY5749 (Strain background described in Tab. 2, medium composition in Tab. 3). The strain were cultivated for 96 h at blue light (using a commercial blue light LED strip with an advertised wavelength of 465-470 nm and a light intensity of 27.3 µmol $s^{-1}$ $m^{-2}$, wavelength distribution shown in Suppl. Fig. S2) and in the dark. Disrupted cells were extracted with n-hexane and analyzed with a Shimadzu Nexis GC 2030, on a Shimadzu SH-Rxi-5MS column (30 m, 0.25 mm, 0.25 µm) and detected by FID (Detailed description of the quantification is listed in the Suppl.). Quantification of 7-pentadecene, 8-heptadecene and 6,9-heptadecadiene were performed according to (Zhou *et al.*, 2016). The concentration of each hydrocarbon is indicated by dots

(in triplicates), the sum of hydrocarbons and the hydrocarbon composition is represented by a bar plot.]

Fig 1

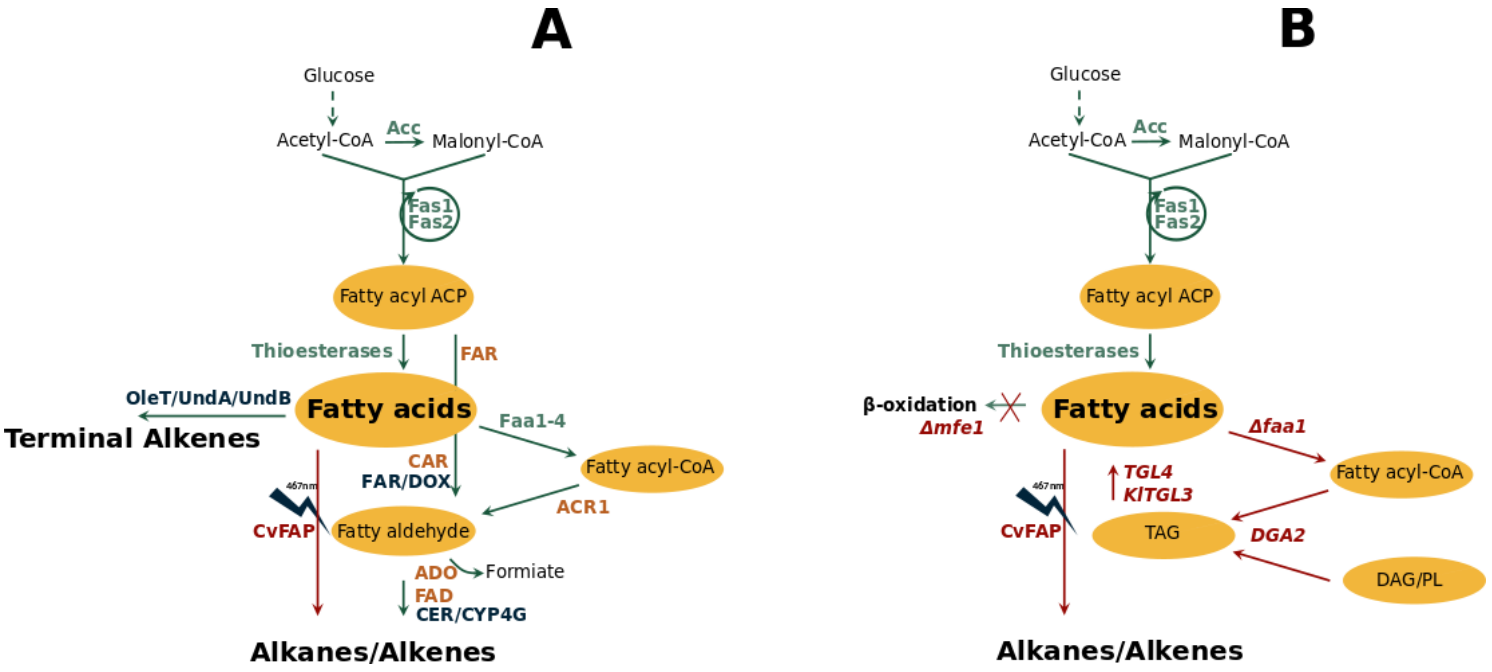
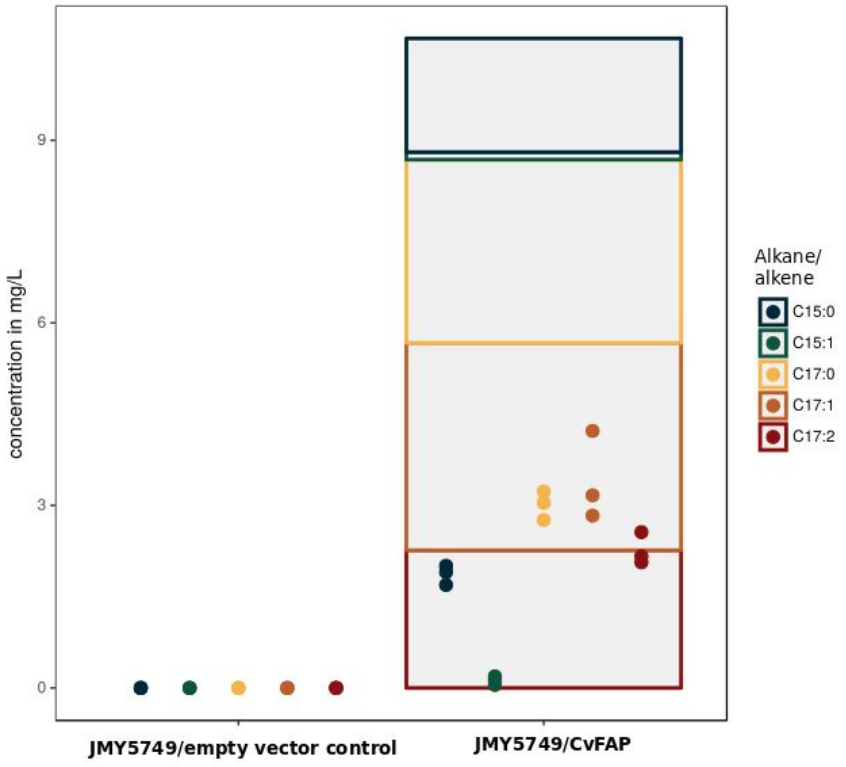



Fig 2




# Supplementary

## Sequence of truncated CvFAP, optimized for *Yarrowia* codon usage

ATGCGAGCCTCTGCTGTCGAGGACATTCGAAAGGTGCTCTCTGACTCCTCTTCCCCCGTCGCCGGACAGAAGTACGACTA
CATCCTGGTCGGAGGCGGTACCGCTGCTTGTGTGCTGGCTAACCGACTCTCCGCCGACGGCTCTAAGCGAGTCCTGGTG
CTGGAGGCTGGTCCTGACAACACCTCCCGAGACGTGAAGATCCCCGCCGCTATTACCCGACTGTTCCGATCTCCCCTGGA
CTGGAACCTCTTCTCTGAGCTGCAGGAGCAGCTCGCTGAGCGACAAATCTACATGGCCCGAGGTCGACTGCTCGGAGGCT
CTTCCGCCACCAACGCTACCCTGTACCACCGAGGCGCCGCTGGTGACTACGACGCTTGGGGAGTCGAGGGCTGGTCTTC
CGAGGACGTCCTCTCCTGGTTCGTGCAGGCCGAGACCAACGCTGACTTCGGACCTGGTGCTTACCACGGCTCTGGTGGA
CCTATGCGAGTCGAGAACCCCCGATACACCAACAAGCAGCTGCACACCGCCTTCTTCAAGGCCGCTGAGGAAGTCGGACT
CACCCCCAACTCCGACTTCAACGACTGGTCTCACGACCACGCTGGTTACGGAACCTTCCAGGTCATGCAGGACAAGGGCA
CCCGAGCCGACATGTACCGACAGTACCTGAAGCCCGTCCTCGGTCGACGAAACCTGCAGGTGCTCACCGGAGCCGCTGT
CACCAAGGTGAACATTGACCAGGCTGCTGGAAAGGCTCAGGCTCTGGGCGTCGAGTTCTCCACCGACGGTCCCACCGGA
GAGCGACTGTCCGCTGAGCTCGCTCCCGGCGGTGAGGTCATTATGTGTGCTGGTGCTGTGCACACCCCCTTCCTGCTCAA
GCACTCTGGTGTGGGTCCTTCTGCTGAGCTGAAGGAGTTCGGTATCCCCGTCGTGTCCAACCTCGCTGGAGTGGGACAGA
ACCTGCAGGACCAGCCTGCTTGTCTCACCGCTGCTCCCGTGAAGGAGAAGTACGACGGAATCGCCATTTCTGACCACATC
TACAACGAGAAGGGCCAGATTCGAAAGCGAGCCATCGCTTCCTACCTGCTCGGAGGCCGAGGTGGACTGACCTCTACCG
GCTGTGACCGAGGTGCCTTCGTCCGAACCGCTGGTCAGGCTCTGCCCGACCTCCAGGTCCGATTCGTGCCTGGAATGGC
TCTCGACCCCGACGGTGTCTCCACCTACGTGCGATTCGCCAAGTTCCAGTCCCAGGGTCTGAAGTGGCCCTCTGGAATTA
CCATGCAGCTCATCGCCTGTCGACCCCAGTCCACCGGCTCTGTGGGTCTGAAGTCTGCCGACCCCTTCGCCCCTCCCAAG
CTCTCTCCTGGATACCTGACCGACAAGGACGGTGCCGACCTGGCTACCCTCCGAAAGGGTATTCACTGGGCTCGAGACGT
GGCTCGATCTTCTGCTCTGTCCGAGTACCTCGACGGAGAGCTGTTCCCCGGTTCCGGAGTCGTGTCTGACGACCAGATCG
ACGAGTACATTCGACGATCTATCCACTCTTCCAACGCTATCACCGGTACCTGTAAGATGGGAAACGCCGGCGACTCTTCCT
CTGTCGTGGACAACCAGCTGCGAGTCCACGGTGTGGAGGGACTCCGAGTCGTGGACGCCTCTGTCGTTCCTAAGATTCCC
GGCGGTCAGACCGGAGCTCCTGTCGTGATGATTGCTGAGCGAGCCGCTGCCCTGCTCACTGGCAAGGCTACTATCGGTG
CTTCCGCCGCCGCTCCTGCTACTGTGGCTGCTTAG

**Fig S1:** Vector map of CvFAP expression plasmid p15018_CvFAP was created by Geneious v.10.2.3. The replicative, centromeric *Y. lipolytica, E. coli* shuttle-plasmid p15018 consists of

CEN1-2, ARS68 region, pUC1 ori, kanamycin, ampicillin resistance cassette, as well as native *pTEF1* promoter and *tXPR2* terminator for gene expression and hphNT2 cassette (HPH) encoding for hygromycin resistance.

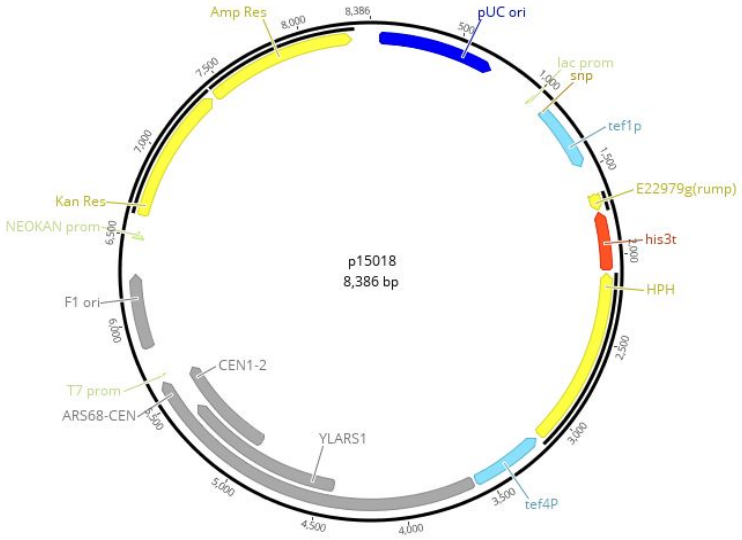
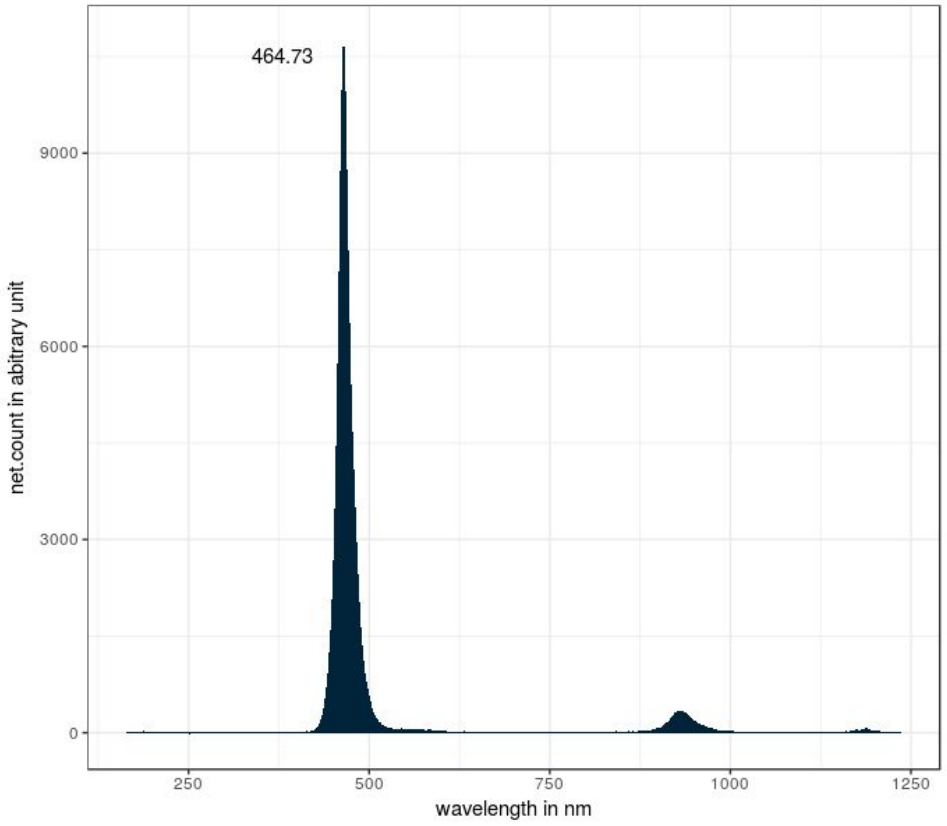



**Fig S2**: Light emission of advertised distinct 465-470 nm LED-light strip by wavelengths. The defined spectrum was verified by broad range spectrometer LR1-T from ASEQ instruments.



**Transformation-associated recombination assisted by *Y. lipolytica* (YaliTAR)**

For time-saving *in vivo* assembly, Yarrowia strain H222 *Δku70* strain was co-transformed (Xuan *et al.*, 1988) with linearized replicative vector p15018 backbone (Fig1, Suppl.), previously digested by MluI and NotI, as well as CvFAP fragment, including 43 bp homologous sequences to pTEF1 promoter (additional 6 bp MluI restriction site for further promoter exchanges) and tXPR2 terminator of p15018. The codon optimized CvFAP fragment was purchased at Baseclear B.V., without predicted targeting sequence as shown in (Sorigué *et al.*, 2017). Oligonucleotides GCAAGGCTACTATCGGTGCT and GGAGGTGTTGTCAGGACCAG were used to amplify the overlapping fragment. Positive clones were selected on YPD2% agar plates including 400 µg/ml Hygromycin after 1-2 days of incubation at 30°C. Vectors, recovered from 4 out of 14 colonies, were further verified by sequencing, whereby 50% showed the correct sequence.

**Yeast strains, cultivation conditions and sampling**

*Yarrowia* strains H222 *Δku70*, S33001 and JMY5749 (Tab. 2) were transformed with p15018_CvFAP vector and corresponding constructs and grown in shake flasks.

5 ml YPD2% (referred elsewhere) for inoculation and 25 ml YSM medium (low mineral salt medium) for the induction of lipid body formation were used.

Cultivations were performed in shake flasks at RT or 28°C and 180 rpm. The shakers were darkened as indicated. Light for the photoenzyme was provided by a commercial blue light LED strip with an advertised wavelength of 465-470 nm, light intensity of 27.3 µmol $s^{-1}$ $m^{-2}$ was determined by using Li-1000 Datalogger LI-COR for PAR (Photosynthetically Active Radiation) range (Fig. S2., Suppl.) or a common LED plant breeding light from Florally Inc. (Shenzhen, Guangdong, 518000,CN). Samples were taken after 96 hours for the determination of cell dry weight, intracellular and extracellular hydrocarbons and metabolites in the supernatant. For analytics, the entire volume of supernatant and cells were utilized.

**Hydrocarbon analytics of cell extract and supernatant**

Cell samples were extracted by simultaneous disruption of cells using a Vortexer (10 min, 3000 rpm) from Heathrow Scientific in 300 µL n-Hexane containing 1.5 µmol n-dodecane as internal standard. Whole supernatant was extracted using 1.8 mL n-hexane containing 1.5 µmol n-dodecane as internal standard. Detection of hydrocarbons was performed using gas chromatography. The samples were analyzed with a Shimadzu Nexis GC 2030, on a Shimadzu SH-Rxi-5MS column (30 m, 0.25 mm, 0.25 µm) and detected by FID. The temperature of inlet and FID were set to 250°C and 310°C, respectively. The linear velocity of hydrogen was set to 50 cm/s. The split was set to 50 for samples of cell extractions and to 10 for samples of the extracted supernatant.

The initial temperature of the column oven was set to 50 °C, which was held for 2.5 min, followed by a ramp to 250 °C at a rate of 10 °C per min, followed by a ramp to 300°C at a rate of 10 °C per min and a final step at 300°C for 10 min. The analytical GC grade standards undecane, tridecane, pentadecane, heptadecane and the C8-C20 alkane standard solution were purchased at Sigma Aldrich. Quantification of 7-pentadecene, 8-heptadecene and 6,9-heptadecadiene were performed according to (Zhou *et al.*, 2016). Corresponding peaks were clearly distinguishable from background noise. For optimisation of hydrocarbon analytics (results in publication Fig. 2), sample volume was reduced to 1 ml and split was set to 5 for samples of cell extraction.

**Fig. S3 AB:** [Assigned peaks of pentadecane (C15:0), 7-pentadecene (C15:1), heptadecane (C17:0) 8-heptadecene (C17:1) and 6,9-heptadecadiene (C17:2) are shown for analysis of cell extraction of alkane producing strain JMY5749/CvFAP in comparison to the empty vector control.]

MS spectra, using Shimadzu GCMS QP2010 and column BPX5 (Column has equal properties to SH-Rxi-5MS, but the retention time was slightly shifted, the program is described above) from selected samples were compared to NIST database (GCMSsolution version 4.42, NIST ) and confirmed presence of saturated pentadecane (97 % similarity), 8-heptadecene (91 % similarity) and heptadecane (93 % similarity). The retention time difference between monounsaturated 1-pentadecene standard (15.969 min, 98 % similarity) and saturated pentadecane (16.049 min) as well as between 8-heptadecene (18.152 min) and heptadecane (18.433 min) further confirmed the above assumed assignments.

**Fig. S4:** [Hydrocarbon production with CvFAP expressed in *Yarrowia* H222 *Δalk1* and JMY5749 (Strain backgrounds described in Tab. 2 of publication, medium composition in Tab. 3 of publication). The strains were cultivated for 96 h at blue light (using a commercial blue light LED strip with an advertised wavelength of 465-470 nm and a light intensity of 27.3 µmol $s^{-1}$ $m^{-2}$, wavelength distribution shown in Suppl. Fig. S2) and in the dark. Disrupted cells were extracted with n-hexane and analyzed with a Shimadzu Nexis GC 2030, on a Shimadzu SH-Rxi-5MS column (30 m, 0.25 mm, 0.25 µm) and detected by FID (Detailed description of the quantification is listed in the Suppl.). Quantification of 7-pentadecene, 8-heptadecene and 6,9-heptadecadiene were performed according to (Zhou *et al.*, 2016). The concentration of each hydrocarbon is indicated by dots (in triplicates), the sum of hydrocarbons and the hydrocarbon composition is represented by a bar plot. A hydrocarbon concentration of 1.5514 +/- 247.8 µg/L was achieved for JMY5749 under exposure of blue light. In contrast, produced amount of hydrocarbons in S33001 background were only 56.0+/-4.0 µg/L (Suppl., Tab S1)]

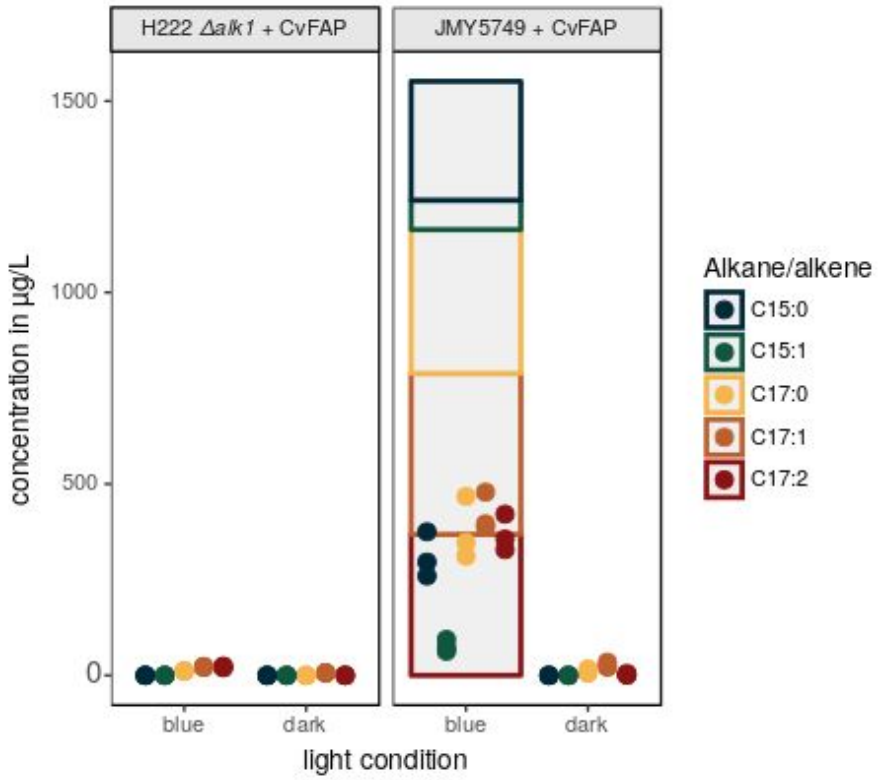
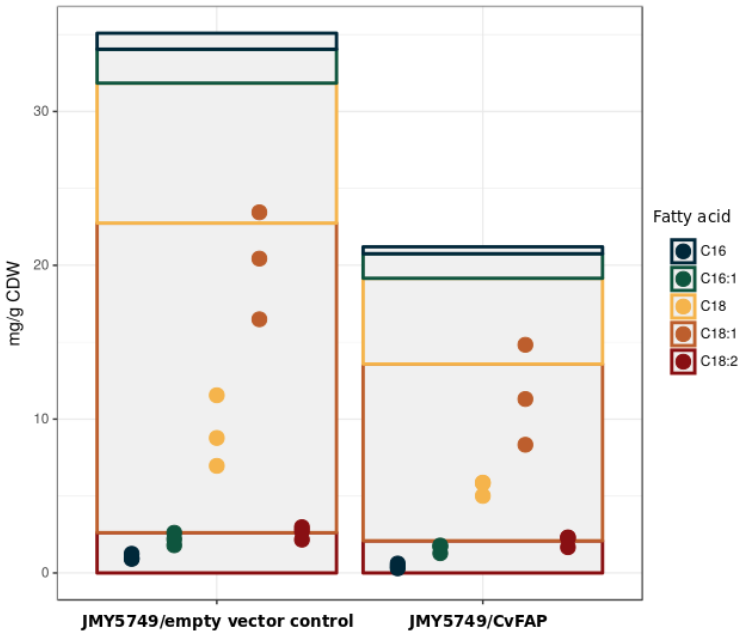



**Fig S5:** [Intracellular fatty acid concentrations of *Yarrowia* JMY5749/CvFAP constructs and empty vector control. Cultivation conditions are listed in publication Fig.2 and above. Description of lipid analytics is mentioned below. The concentration of each fatty acid is indicated by dots (in triplicates), the sum (medium) of fatty acids and the fatty acid composition is represented by a bar plot. Total fatty acid concentration was significant lower (Students two Sample t-test: t=3.2778, df=4, p-value=0.03056) for JMY5749/CvFAP construct (21.20272+/-4.396019) in comparison to empty vector control (35.08924+/-5.875347). ]




**Lipid analytics of cell extract**

Samples of 1 ml volume were extracted by simultaneous disruption of cells using Retsch Mixer Mill MM 400 (20 min, frequence of 30/s at RT) in 300 µL hexane/2-propanol (3:1, v/v) containing internal standard (5 mM tridecanoic acid) and 200 µl A. dest. After phase separation, direct transesterification was carried out by adding 500 µl 2% (w/v) sulfuric acid in methanol and subsequent incubation at 60 °C for 2 h & 14000 rpm in a thermomixer. Fatty acid methyl esters (FAME) were extracted by 300 µl hexane after centrifugation step. In opposite to hydrocarbon analytics, GC program was changed as listed below. For peak assignment, FAME mix from Sigma Aldrich (CRM18918) were used. For quantification, corresponding single FAMEs from Sigma Aldrich Fluka in the concentration range of 0.025 - 8 mM were measured. Data were processed using LabSolutions 5.92 and R version 3.4.4 (2018-03-15).

Column oven temperature program:

Temp. 90 °C, hold 5 min

Rate 15, final temp. 190 °C

Rate 2.0, final temp. 200 °C, hold 1 min

Rate 0.5, final temp. 202.5 °C, hold 1 min

Rate 20, final temp. 300 °C, hold 5 min

**Tab S1:**

| Variant | Light condition | Alkane/Alkene | Mean amount per cdw µg/mg | Standard deviation amount per cdw µg/mg | Mean amount per culture volume µg/L | Standard deviation amount per culture volume µg/L |
|---|---|---|---|---|---|---|
| H222 Δku70 + CvFAP | blue | C15:1 | 0.000000 | 0.000000 | 0.0 | 0.0 |
| H222 Δku70 + CvFAP | dark | C15:0 | 0.000000 | 0.000000 | 0.0 | 0.0 |
| H222 Δku70 + CvFAP | dark | C15:1 | 0.000000 | 0.000000 | 0.0 | 0.0 |
| H222 Δku70 + CvFAP | dark | C17:0 | 0.000000 | 0.000000 | 0.0 | 0.0 |
| H222 Δku70 + CvFAP | dark | C17:2 | 0.000000 | 0.000000 | 0.0 | 0.0 |
| H222 Δku70 + CvFAP | dark | C17:1 | 0.001268 | 0.000737 | 1.5 | 0.9 |
| H222 Δku70 + CvFAP | blue | C15:0 | 0.010064 | 0.005915 | 10.9 | 7.1 |
| H222 Δku70 + CvFAP | blue | C17:2 | 0.019164 | 0.009226 | 19.7 | 9.2 |
| H222 Δku70 + CvFAP | blue | C17:1 | 0.031145 | 0.012653 | 31.9 | 13.0 |
| H222 Δku70 + CvFAP | blue | C17:0 | 0.048694 | 0.010288 | 49.6 | 5.9 |
| H222 Δku70 + empty vector control | blue | C15:0 | 0.000000 | 0.000000 | 0.0 | 0.0 |
| H222 Δku70 + empty vector control | blue | C15:1 | 0.000000 | 0.000000 | 0.0 | 0.0 |
| H222 Δku70 + empty vector control | blue | C17:0 | 0.000000 | 0.000000 | 0.0 | 0.0 |
| H222 Δku70 + empty vector control | blue | C17:2 | 0.000000 | 0.000000 | 0.0 | 0.0 |
| H222 Δku70 + empty vector control | dark | C15:0 | 0.000000 | 0.000000 | 0.0 | 0.0 |
| H222 Δku70 + empty vector control | dark | C15:1 | 0.000000 | 0.000000 | 0.0 | 0.0 |

| | | | | | | |
|---|---|---|---|---|---|---|
| H222 Δku70 + empty vector control | dark | C17:0 | 0.000000 | 0.000000 | 0.0 | 0.0 |
| H222 Δku70 + empty vector control | dark | C17:2 | 0.000000 | 0.000000 | 0.0 | 0.0 |
| H222 Δku70 + empty vector control | blue | C17:1 | 0.001751 | 0.002891 | 1.5 | 2.4 |
| H222 Δku70 + empty vector control | dark | C17:1 | 0.005416 | 0.007681 | 5.5 | 8.0 |
| JMY5749 + CvFAP (non- optimized extraction method) | dark | C15:0 | 0.000000 | 0.000000 | 0.0 | 0.0 |
| JMY5749 + CvFAP(non-optimized extraction method) | dark | C15:1 | 0.000000 | 0.000000 | 0.0 | 0.0 |
| JMY5749 + CvFAP(non-optimized extraction method) | dark | C17:2 | 0.000746 | 0.000659 | 2.6 | 2.3 |
| JMY5749 + CvFAP(non-optimized extraction method) | dark | C17:0 | 0.002857 | 0.001751 | 10.1 | 6.1 |
| JMY5749 + CvFAP(non-optimized extraction method) | dark | C17:1 | 0.007524 | 0.002087 | 26.7 | 7.1 |
| JMY5749 + CvFAP(non-optimized extraction method) | blue | C15:1 | 0.027968 | 0.008552 | 76.7 | 15.7 |
| JMY5749 + CvFAP(non-optimized extraction method) | blue | C15:0 | 0.113092 | 0.033040 | 310.4 | 58.9 |
| JMY5749 + CvFAP(non-optimized extraction method) | blue | C17:2 | 0.132950 | 0.027600 | 368.5 | 47.6 |
| JMY5749 + CvFAP(non-optimized extraction method) | blue | C17:0 | 0.136845 | 0.043067 | 375.4 | 81.9 |
| JMY5749 + CvFAP(non-optimized extraction method) | blue | C17:1 | 0.151946 | 0.032313 | 420.4 | 50.6 |
| H222 Δalk1 + CvFAP | blue | C15:0 | 0.000000 | 0.000000 | 0.0 | 0.0 |
| H222 Δalk1 + CvFAP | blue | C15:1 | 0.000000 | 0.000000 | 0.0 | 0.0 |
| H222 Δalk1 + CvFAP | dark | C15:0 | 0.000000 | 0.000000 | 0.0 | 0.0 |
| H222 Δalk1 + CvFAP | dark | C15:1 | 0.000000 | 0.000000 | 0.0 | 0.0 |

| | | | | | | |
|---|---|---|---|---|---|---|
| H222 Δalk1 + CvFAP | dark | C17:0 | 0.000000 | 0.000000 | 0.0 | 0.0 |
| H222 Δalk1 + CvFAP | dark | C17:2 | 0.000000 | 0.000000 | 0.0 | 0.0 |
| H222 Δalk1 + CvFAP | dark | C17:1 | 0.001873 | 0.000055 | 6.7 | 0.4 |
| H222 Δalk1 + CvFAP | blue | C17:0 | 0.003524 | 0.000326 | 11.7 | 0.8 |
| H222 Δalk1 + CvFAP | blue | C17:2 | 0.006632 | 0.000795 | 22.1 | 2.3 |
| H222 Δalk1 + CvFAP | blue | C17:1 | 0.006670 | 0.000617 | 22.2 | 1.4 |
| JMY5749 + empty vector control (non-optimized extraction method) | blue | C15:0 | 0.000000 | 0.000000 | 0.0 | 0.0 |
| JMY5749 + empty vector control (non-optimized extraction method) | blue | C15:1 | 0.000000 | 0.000000 | 0.0 | 0.0 |
| JMY5749 + empty vector control (non-optimized extraction method) | blue | C17:0 | 0.000000 | 0.000000 | 0.0 | 0.0 |
| JMY5749 + empty vector control (non-optimized extraction method) | blue | C17:1 | 0.000000 | 0.000000 | 0.0 | 0.0 |
| JMY5749 + empty vector control (non-optimized extraction method) | blue | C17:2 | 0.000000 | 0.000000 | 0.0 | 0.0 |
| JMY5749 + empty vector control (non-optimized extraction method) | dark | C15:0 | 0.000000 | 0.000000 | 0.0 | 0.0 |
| JMY5749 + empty vector control (non-optimized extraction method) | dark | C15:1 | 0.000000 | 0.000000 | 0.0 | 0.0 |
| JMY5749 + empty vector control (non-optimized extraction method) | dark | C17:0 | 0.000000 | 0.000000 | 0.0 | 0.0 |

| | | | | | | |
|---|---|---|---|---|---|---|
| JMY5749 + empty vector control (non-optimized extraction method) | dark | C17:2 | 0.000000 | 0.000000 | 0.0 | 0.0 |
| JMY5749 + empty vector control (non-optimized extraction method) | dark | C17:1 | 0.000031 | 0.000049 | 0.1 | 0.2 |
| H222 Δalk1 + empty vector control | blue | C15:0 | 0.000000 | 0.000000 | 0.0 | 0.0 |
| H222 Δalk1 + empty vector control | blue | C15:1 | 0.000000 | 0.000000 | 0.0 | 0.0 |
| H222 Δalk1 + empty vector control | blue | C17:0 | 0.000000 | 0.000000 | 0.0 | 0.0 |
| H222 Δalk1 + empty vector control | blue | C17:1 | 0.000000 | 0.000000 | 0.0 | 0.0 |
| H222 Δalk1 + empty vector control | blue | C17:2 | 0.000000 | 0.000000 | 0.0 | 0.0 |
| H222 Δalk1 + empty vector control | dark | C15:0 | 0.000000 | 0.000000 | 0.0 | 0.0 |
| H222 Δalk1 + empty vector control | dark | C15:1 | 0.000000 | 0.000000 | 0.0 | 0.0 |
| H222 Δalk1 + empty vector control | dark | C17:0 | 0.000000 | 0.000000 | 0.0 | 0.0 |
| H222 Δalk1 + empty vector control | dark | C17:2 | 0.000000 | 0.000000 | 0.0 | 0.0 |
| H222 Δalk1 + empty vector control | dark | C17:1 | 0.000168 | 0.000174 | 0.6 | 0.6 |

## Tab S2:

| Variant | Light | Mean cdw g/mL | Standard deviation cdw g/mL | Mean consumed glucose g/L | Standard deviation consumed glucose g/L |
|---|---|---|---|---|---|
| JMY5749 + cvFAP (non-optimized extraction method) | blue | 0.0036 | 0.0005 | 15.6 | 11.1 |
| JMY5749 + cvFAP (non-optimized | dark | 0.0040 | 0.0002 | 19.4 | 2.3 |

| | | | | | |
|---|---|---|---|---|---|
| extraction method) | | | | | |
| H222 Δalk1 + cvFAP | blue | 0.0040 | 0.0005 | 16.3 | 5.6 |
| H222 Δalk1 + cvFAP | dark | 0.0044 | 0.0008 | 20.9 | 5.9 |
| JMY5749 + empty vector control (non-optimized extraction method) | blue | 0.0049 | 0.0006 | 10.6 | 5.0 |
| JMY5749 + empty vector control (non-optimized extraction method) | dark | 0.0047 | 0.0007 | 13.3 | 3.8 |
| H222 Δalk1 + empty vector control | blue | 0.0034 | 0.0004 | 23.8 | 2.4 |
| H222 Δalk1 + empty vector control | dark | 0.0069 | 0.0016 | 11.4 | 11.8 |

## References

Zhou, Y.J., Buijs, N.A., Zhu, Z., Gómez, D.O., Boonsombuti, A., Siewers, V., and Nielsen, J. (2016) Harnessing yeast peroxisomes for biosynthesis of fatty-acid-derived biofuels and chemicals with relieved side-pathway competition. *J. Am. Chem. Soc.* **138:** 15368–15377.