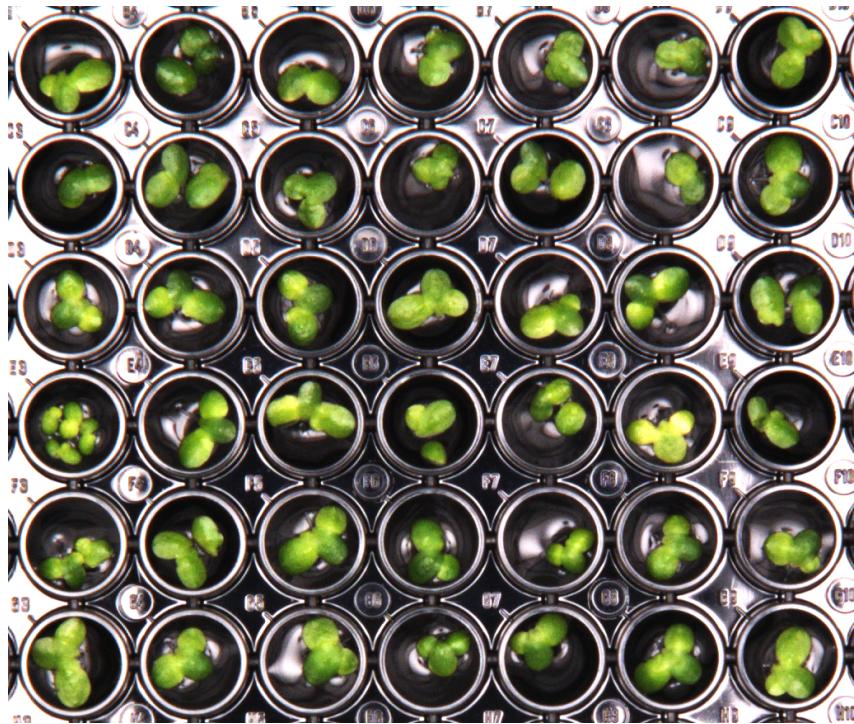


Engineering Triacylglycerol Accumulation in Duckweed (*Lemna japonica*)

Supplemental materials

(a)



(b)

WT	EV (0-1)	EV (0-2)	EV (0-3)	EV (0-4)	EV (0-5)	WT
EV (0-6)	O (6-1)	O (6-2)	O (6-3)	O (6-4)	O (6-5)	W (22-1)
W (22-2)	W (22-3)	W (22-4)	WT	W (22-7)	W (22-9)	W (22-)10
D (24-1)	D (24-2)	D (24-3)	WT	D (24-4)	OW (25-1)	OW (25-2)
OW (25-3)	OW (25-7)	OD (27-2)	OD (27-3)	OD (27-4)	WD (31-1)	WD (31-3)
WT	OWD (33-1)	OWD (33-3)	OWD (33-4)	OWD (33-5)	OWD (33-6)	WT

Figure S1 Representative images of duckweed. (a) Different lines of duckweed in 96-well plate ready for fluorescence analysis using plate reader. (b) Names of lines in wells of the plate in (a). The number before dash line are represent as: 0 is for *EV*, 6 is for *SiOLE(*)*, 22 is for *CFP-AtWRI1*, 24 is for *MmDGAT2*, 25 is for *SiOLE(*)+CFP-AtWRI1*, 27 is for *SiOLE(*)+MmDGAT*, 31 is for *CFP-AtWRI1+MmDGAT*, 33 is for *SiOLE(*)+CFP-AtWRI1+MmDGAT*. Different lines were also indicated by different colors.

(a)

WT	0-1	0-2	0-3	0-4	0-5	WT
0-6	6-1	6-2	6-3	6-4	6-5	22-1
22-2	22-3	22-4	WT	22-7	22-9	22-10
24-1	24-2	24-3	WT	24-4	25-1	25-2
25-3	25-7	27-2	27-3	27-4	31-1	31-3
WT	33-1	33-3	33-4	33-5	33-6	WT

(b)

Frond No.	Constructs
6	L2-0 (EV): <i>BAR+EYFP</i>
5	L2-6 (O): <i>BAR+SiOLE(*)+EYFP</i>
7	L2-22 (W): <i>BAR+XVE:CFP-AtWRI1+EYFP</i>
4	L2-24 (D): <i>BAR+MmDGAT+EYFP</i>
4	L2-25 (OW): <i>BAR+XVE:CFP-AtWRI1+SiOLE(*)+EYFP</i>
3	L2-27 (OD): <i>BAR+SiOLE(*)+MmDGAT+EYFP</i>
2	L2-31 (WD): <i>BAR+XVE:CFP-AtWRI1+MmDGAT+EYFP</i>
5	L2-33 (OWD): <i>BAR+XVE:CFP-AtWRI1+SiOLE(*)+MmDGAT+EYFP</i>

133 530 20,000

Fluorescence intensity (A.U.)

Figure S2 Screening of *L. japonica* transformants using plate reader. (a) Fluorescence intensity of EYFP showing by gradient color from blue-white-red. (b) Numbers of positive frond transformed with different constructs as indicated by YFP fluorescence.

Abbreviations: WT, Wilde type (*Lemna japonica* 8627 or *Lj8627*); A.U., Arbitrary units. Zm, *Z. mays*; At, *A. thaliana*; Si, *S. indicum*; Mm, *M. musculus*. *BAR*, Phosphinothricin acetyl transferase; *EYFP*, Enhanced yellow fluorescent protein.

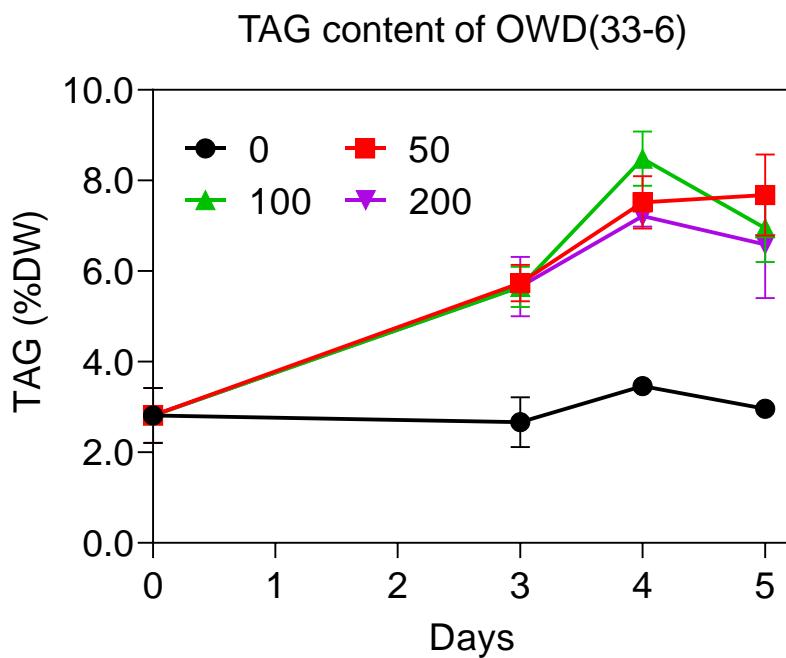


Figure S3 TAG accumulation in duckweed treated with different concentrations of estradiol for different time periods. OWD (33-6) (*(SiOLE(*)+XVE:CFP-AtWRI1+MmDGAT2)*) transgenic line was used in this experiment. Fronds were cultivated in half-strength SH medium (pH 5.6) under continuous cool white fluorescent light illumination (approximate 100 $\mu\text{mol}\cdot\text{m}^{-2}\text{s}^{-1}$) at 23 °C with ambient CO₂ level.

Data are means of three biological replicates and error bars indicate standard deviations. Significant differences between groups ($P < 0.05$) as identified by one-way ANOVA and Tukey's multiple comparisons test are shown by different letters.

(a)

A 154 T 119 C 233 G 181 | GC%: 60% | Length: 687

>CFP

TTCTCGAGGCCG**ATG**GCCCTGTCCAACAAGTTCATGGGGACGACATG
AAGATGACGTACCACATGGACGGTGCCTGAACGGGCACTACTTCACC
GTGAAGGGCGAAGGCTCCGGGAAGCCGTATGAAGGGACCCAGACCTC
CACCTTCAAGGTGACCATGGCCAATGGGGGCCCCCTCGCTTCAGCTT
CGACATCCTCAGCACCGTGTTCATGTACGGAACAGATGCTTCACCGCC
TACCCGACGAGCATGCCGATTACTTCAAGCAAGCCTCCCCGACGGC
ATGTCCTACGAACGCACCTCACCTACGAAGATGGCGGGTGGCTACC
GCTAGCTGGGAGATCTCCCTCAAGGGCAACTGCTTCGAGCACAAGTCC
ACCTTCCACGGCGTCAATTCCCCGCTGACGGCCCCGTGATGGCCAAG
AAAACCACCGGCTGGGACCCCAGCTCGAGAAGATGACCGTGTGCGAC
GGCATTCTGAAGGGCGACGTACCGCTTCATGCTCCAAGGCAGGG
GGCAACTACCGCTGCCAGTTCCATACCTCCTACAAGACGAAGAAGCCC
GTGACCATGCCCGAACACGTGGTCGAGCACCGCATGCCAGAACCC
GATCTCGATAAGGGCGGCAACTCCGTGCAACTACCGAGCAGCAGCTGTG
GCTCACATCACCTCCGTCCCCCTTC

(b)

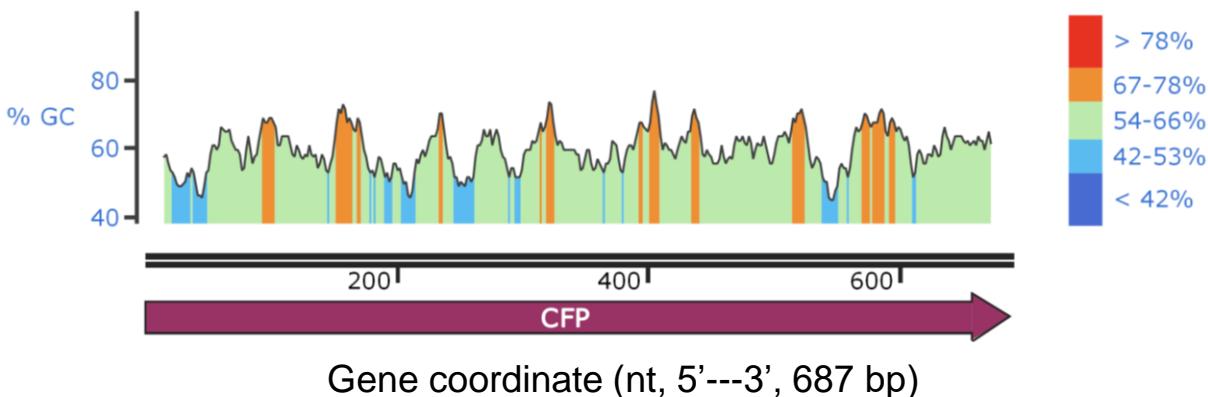


Figure S4 Sequence and feature of optimized *CFP*. (a) gene sequence of *CFP* without stop codon, the start codon ATG showing as orange color, with N-terminal fusion of Kozak sequence. Exempt the Kozak sequence and stop codon, the total length of *CFP* is 687 bp, with 60% of average GC content. (b) Distribution of GC content in *CFP* scanned by 25-nt sliding windows. The total range for colors are 37%, and the midpoint for colors were 60%.

Table S1: TAG content as percent (DW) in WT and different transgenic lines of *Lemna japonica*. D, DGAT1; EV, empty vector; W, WRI1. The values shown for each biological replicate are averages for two independent transgenic lines.

Biological replicate	Genotype / condition							
	EV	O	W	D	OW	OD	WD	OWD
A1	0.08	0.10	0.57	0.58	0.63	3.41	1.50	6.99
A2	0.08	0.10	0.45	0.53	0.50	3.06	1.24	6.77
A3	0.09	0.10	0.70	0.63	0.76	3.76	1.76	7.22
B1	0.08	0.09	0.47	0.20	0.55	3.86	2.59	8.68
B2	0.07	0.08	0.47	0.18	0.51	3.65	2.14	8.13
B3	0.09	0.11	0.48	0.22	0.59	4.07	3.03	9.24
Average ± standard deviation	0.08 ± 0.01	0.10 ± 0.01	0.52 ± 0.10	0.39 ± 0.21	0.59 ± 0.10	3.64 ± 0.36	2.04 ± 0.68	7.84 ± 1.00