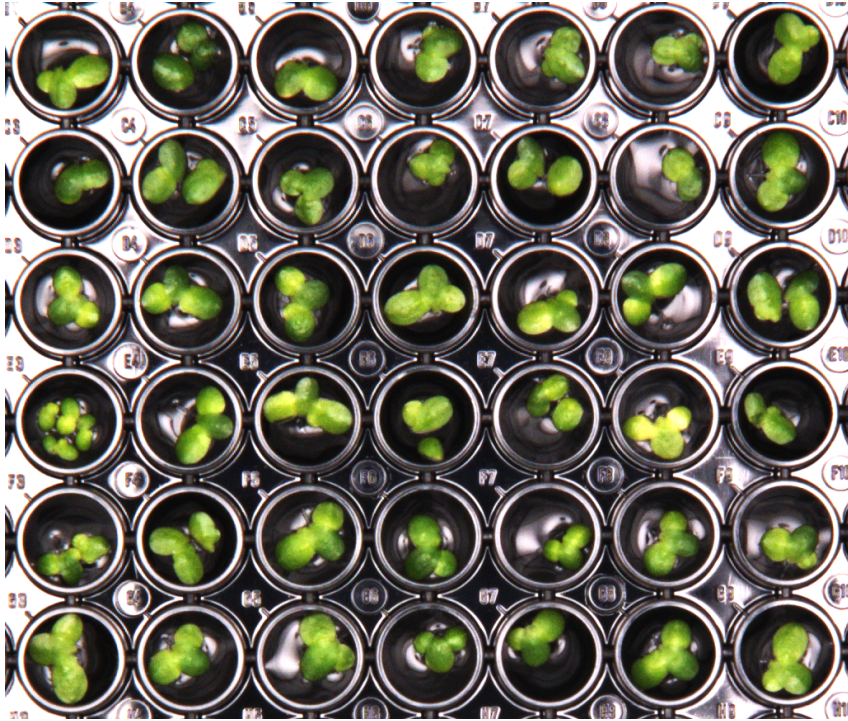


**Engineering Triacylglycerol
Accumulation in Duckweed (*Lemna
japonica*)**

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-AtWR11*, 24 is for *MmDGAT2*, 25 is for *SiOLE*(*)+*CFP-AtWR11*, 27 is for *SiOLE*(*)+*MmDGAT*, 31 is for *CFP-AtWR11*+*MmDGAT*, 33 is for *SiOLE*(*)+*CFP-AtWR11*+*MmDGAT*. Different lines were also indicated by different colors.

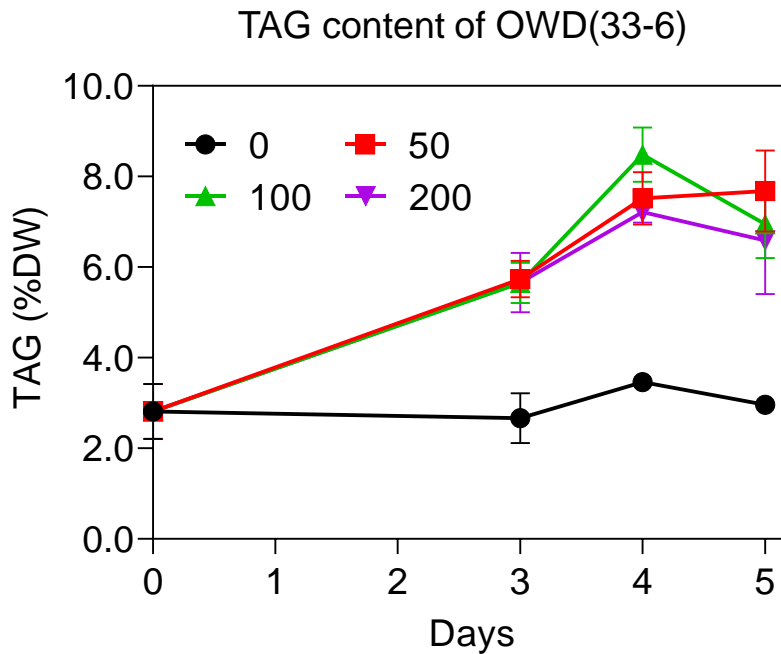


Figure S3 TAG accumulation in duckweed treated with different concentrations of estradiol for different time periods. OWD (33-6) ((*SiOLE*(*)+*XVE:CFP-AtWRI1*+*MmDGAT2*) transgenic line was used in this experiment. Fronds were cultivated in half-strength SH medium (pH 5.6) under continuous cool white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

```
TTCTCGAGGCCGATGGCCCTGTCCAACAAGTTCATCGGGGACGACATG
AAGATGACGTACCACATGGACGGGTGCGTGAACGGGCACTACTTCACC
GTGAAGGGCGAAGGCTCCGGGAAGCCGTATGAAGGGACCCAGACCTC
CACCTTCAAGGTGACCATGGCCAATGGGGGGCCCTCGCTTTCAGCTT
CGACATCCTCAGCACCGTGTTTCATGTACGGGAACAGATGCTTCACCGCC
TACCCGACGAGCATGCCCGATTACTTCAAGCAAGCCTTCCCCGACGGC
ATGTCCTACGAACGCACCTTCACCTACGAAGATGGCGGGGTGGCTACC
GCTAGCTGGGAGATCTCCCTCAAGGGCAACTGCTTCGAGCACAAGTCC
ACCTTCCACGGCGTCAATTTCCCCGCTGACGGCCCCGTGATGGCCAAG
AAAACCACCGGCTGGGACCCCAGCTTCGAGAAGATGACCGTGTGCGAC
GGCATTCTGAAGGGCGACGTCACCGCTTTCCTCATGCTCCAAGGCGGG
GGCAACTACCGCTGCCAGTTCCATACCTCCTACAAGACGAAGAAGCCC
GTGACCATGCCCCCGAACCACGTGGTTCGAGCACCGCATCGCCAGAACC
GATCTCGATAAGGGCGGCAACTCCGTGCAACTCACCGAGCACGCTGTG
GCTCACATCACCTCCGTCGTCCCCTTC
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(b)

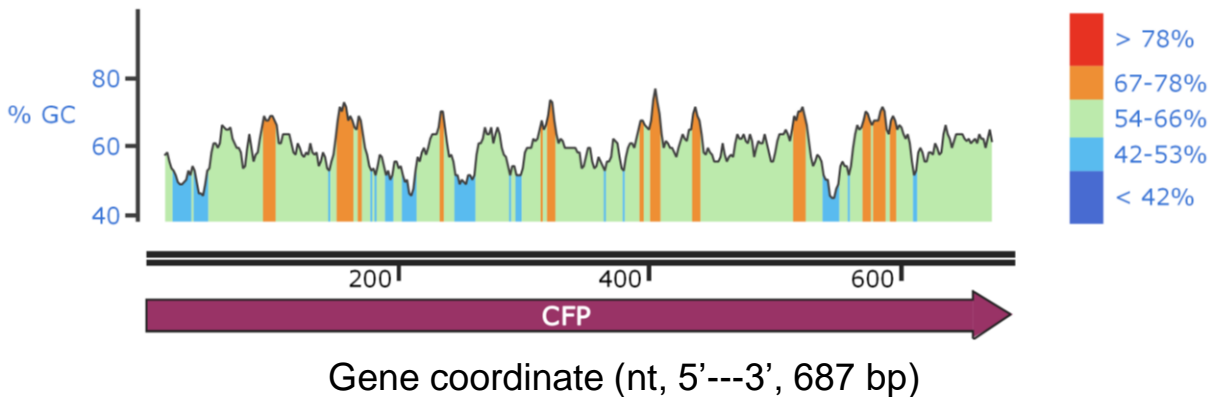


Figure S4 Sequence and feature of optimized *CFP*. (a) gene sequence of *CFP* without stop codon, the start codon ATG showing as orange color, with N-terminal fusion of Kozak sequence. Exempt the Kozak sequence and stop codon, the total length of *CFP* is 687 bp, with 60% of average GC content. (b) Distribution 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 \pm standard deviation	0.08 \pm 0.01	0.10 \pm 0.01	0.52 \pm 0.10	0.39 \pm 0.21	0.59 \pm 0.10	3.64 \pm 0.36	2.04 \pm 0.68	7.84 \pm 1.00