

Additional file

1 **Increased ethylene production by overexpressing phosphoenolpyruvate carboxylase in the**
2 **cyanobacterium *Synechocystis* PCC 6803**

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10 Supplementary material

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12 Table S1. Primers used to amplify the ethylene forming enzyme (*efe*), *phosphoenolpyruvate synthase* (PPSA)
 13 and *phosphoenolpyruvate carboxylase* (PEPc) from *Synechocystis* PCC 6803 (subscript (6803) and
 14 *Synechococcus* PCC 7002 (subscript (7002)). The forward primer (operon) has a RBS upstream of the starting
 15 codon in order to be placed in an operon after the *phosphoenolpyruvate synthase* (PPSA). PPSA forward
 16 primer contains a Flag tag (underlined).

Gene	Forward primer	Reverse primer
Efe	CTCATCTAGAATGACCAATT TGCAAACCTTTTG	CAATGGATCCCTTATTTATC ATCATCATCTTTGTAATC
PEPc	GTCTTCTAGAATGAACTTGG CAGTTCCTG	CGTACTGCAGGGATCCACTA GTTCAACCAGTATTACGCAT TC
PPSA	GTCTTCTAGAATGTCAGGCT <u>CTGACTACAAGGATGACGA</u> <u>TGACAAGGTAAGTTCAGTC</u> GTCGAAAAAA	CGTACTGCAGGGATCCACTA GTCTAGCCTAGGGCTTTTTC C
PEPc-operon	GTCTTCTAGATAGTGGAGGT TAGAGAATGAACTTGGCAG TTCCTG	CGTACTGCAGGGATCCACTA GTTCAACCAGTATTACGCAT TC
PEPc ₇₀₀₂	GTCTTCTAGAATGAACCAAG TCATGCATCCC	CGTACTGCAGGGATCCACTA GTTCAACCCGTGTTCCGCAT
PPSA ₇₀₀₂	GTCTTCTAGAATGTCAGGCT <u>CTGACTACAAGGATGACGA</u> <u>TGACAAGGTTAGCACTCTTA</u> ACACGGC	CGTACTGCAGGGATCCACTA GTCTATCGACCGAGTTTTG TT
PEPc ₇₀₀₂ -operon	GTCTTCTAGATAGTGGAGGT TAGAGAATGAACCAAGTCA TGCATCCC	CGTACTGCAGGGATCCACTA GTTCAACCCGTGTTCCGCAT

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PPSA PCC 6803	MSGSDYKDDKDDVSVVSVKTSVAHKEETALVWFEEVTHDVLGVGGKNSL	146
PPSA PCC 7002	MSGSDYKDDKDDVSVVSVKTSVAHKEETALVWFEEVTHDVLGVGGKNSL	160
PPSA PCC 6803	--GVDVAVRSSATAEDLPEASFAQQGYTYLNVHSLCVLESCHCKFASL	304
PPSA PCC 7002	AHNVDAVAVRSATAEDLPEASFAQQGYTYLNVHSGVGLAECHCKFASL	320
PPSA PCC 6803	NVEVAEPEREKYCINDEELQLARWACIEIDHYSYGVVYTPMDIEAWK	460
PPSA PCC 7002	NVDVPEALQKFCVSDIEDVTLARWATTEIHSYNSVGGYTPMDIEAWK	484
PPSA PCC 6803	AIVGCGDATDTIKTGEDVTICSEGDGVSYGILNVYHETLSNLPRTKI	624
PPSA PCC 7002	AVVGCADATLTKVSGQDVATSCCEGDGKIEYGLKFEVETLSLEMLPT	640
PPSA PCC 6803	LGRQGFEPKEENPMIGWAGSRYYDPNRYEAYALECQALKVRDEMLN	784
PPSA PCC 7002	LGRQGFEPTEENPMIGWAGSRYYDPNRYEAYALECQALKVRDEMLN	800
PPSA PCC 6803	CGQAPSDYPFAEFLVELGDISISLNPDVSLKTVLRAIEVALEKLG	829
PPSA PCC 7002	CGQAPSDYPFAEFLVELGDISISLNPDVSLKTKLTAIAALEQKLGR	846

Figure S1. Sequence alignment of Phosphoenolpyruvate synthase from *Synechocystis* (PPSA PCC 6803) and *Synechococcus* (PPSA PCC 7002). An * indicates positions which have a single, fully conserved residue, : indicates conservation between groups of strongly similar properties - scoring > 0.5 in the Gonnet PAM 250 matrix, . indicates conservation between groups of weakly similar properties - scoring ≤ 0.5 in the Gonnet PAM 250 matrix.