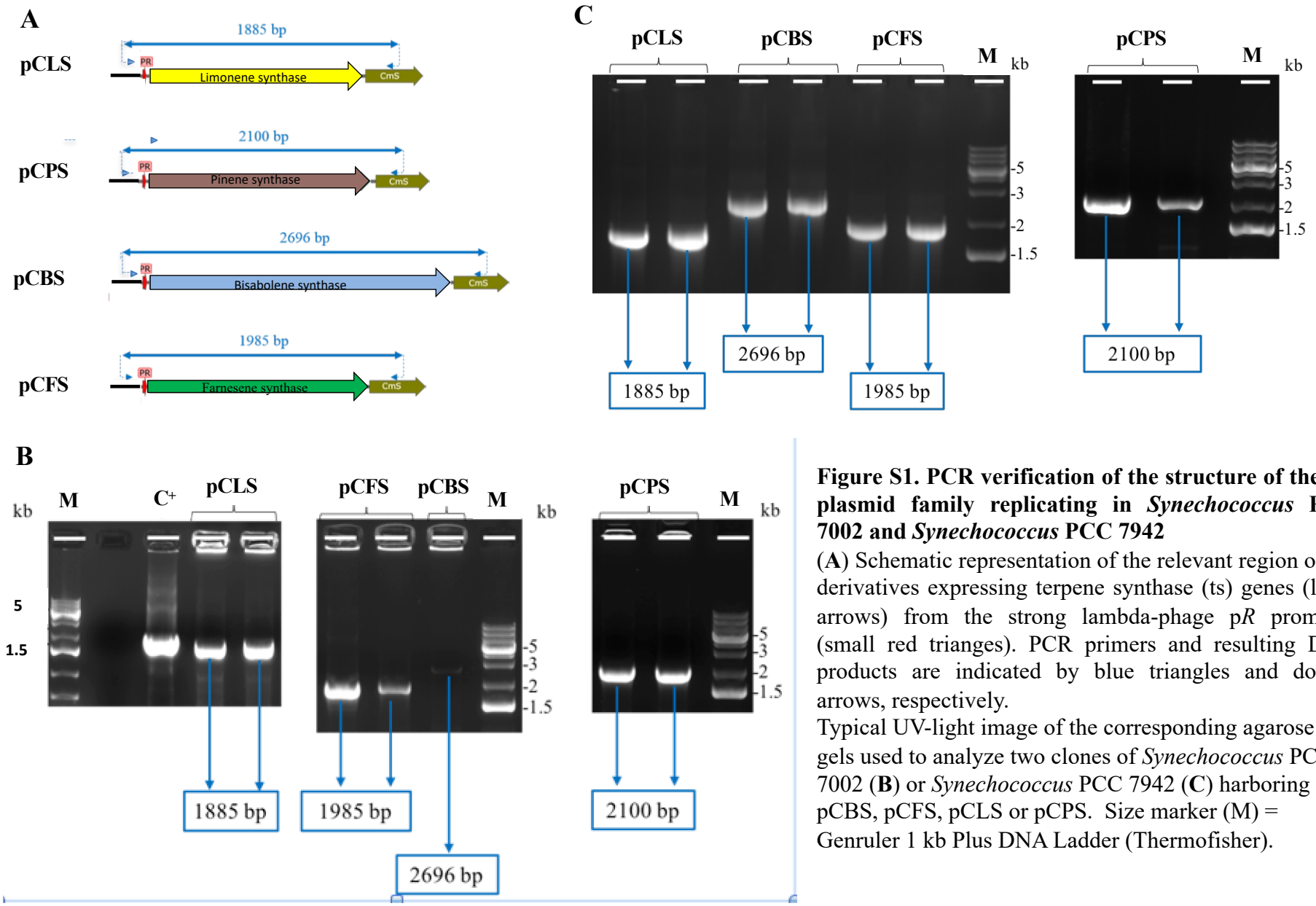


Figure S1. PCR verification of the structure of the pC plasmid family replicating in *Synechococcus* PCC 7002 and *Synechococcus* PCC 7942

(**A**) Schematic representation of the relevant region of pC derivatives expressing terpene synthase (ts) genes (large arrows) from the strong lambda-phage p*R* promoter (small red trianges). PCR primers and resulting DNA products are indicated by blue triangles and double arrows, respectively.

Typical UV-light image of the corresponding agarose gels used to analyze two clones of *Synechococcus* PCC 7002 (**B**) or *Synechococcus* PCC 7942 (**C**) harboring pCBS, pCFS, pCLS or pCPS. Size marker (M) = Genruler 1 kb Plus DNA Ladder (Thermofisher).

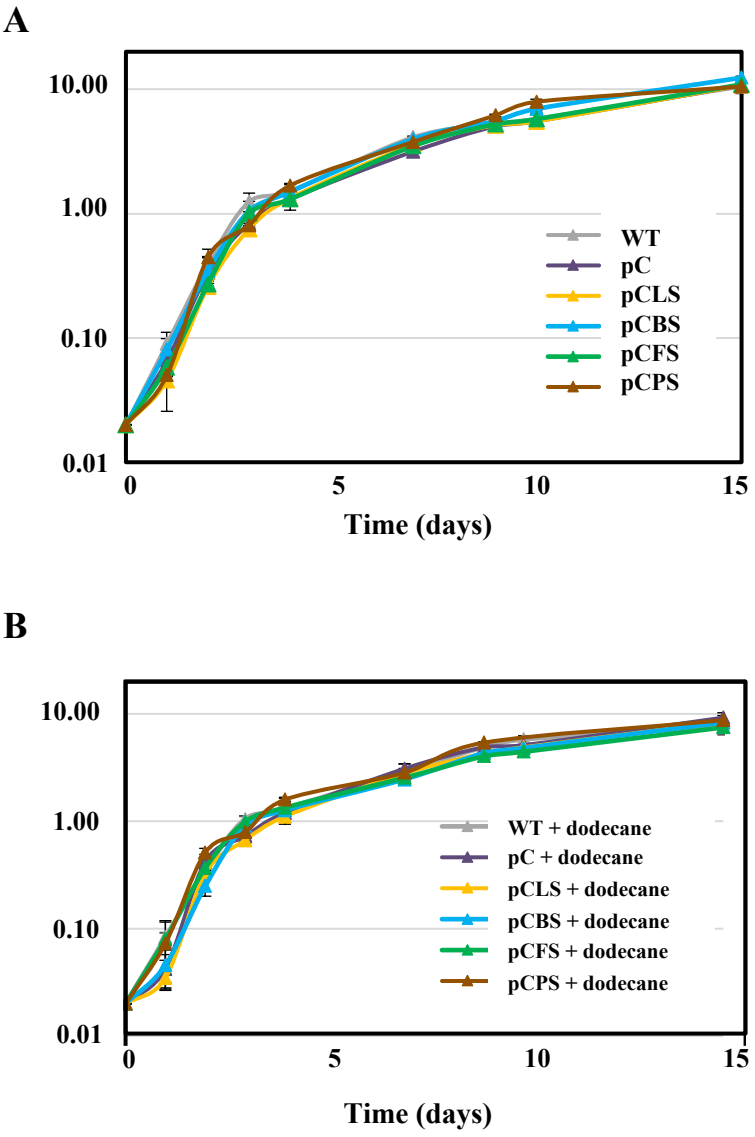


Figure S2. Influence of dodecane and pC-derived plasmids on the photoautotrophic growth of of *Synechococcus* PCC 7002

All strains were cultivated under standard light (2500 lux) and temperature (30°C) in absence (**A**) or presence (**B**) of a dodecane overlay: wild-type (**WT**), or its derivatives carrying the **empty pC vector**, or a pC-derived plasmid expressing the limonene synthase (**pCLS**), bisabolene synthase (**pCBS**), farnesene synthase (**pCFS**) or pinene synthase (**pCPS**). Errors bars represent standard deviation from biological triplicates.

Figure S3A. GC–MS analyses of the dodecane overlays of cultures of the *Synechoccocus* PCC 7002 strains harboring the plasmids pC, pCBS or pCFS

Ion chromatograms (left panels) and corresponding mass spectra (right panels) of farnesene-isomers standards containing bisabolene (**a**) or dodecane samples from cultures of *Synechococcus* PCC 7002 propagating pCFS (**b**, production of α-farnesene), pCBS (**c**, E-α-bisabolene production) or pC (**d**, negative control). β-caryophyllene (retention time = 17.3 min) was used as the fixed-concentration (0.01 g/L) internal standard (IS) for quantification. The concentrations of bisabolene and farnesene were calculated using the standard curves that we have reported during our recent analysis of the production of bisabolene and farnesene by the other model cyanobacterium *Synechocystis* PCC 6803 (See Blanc-Garin *et al* 2022 Biotechnology for Biofuels and Bioproducts 15 https://doi.org/10.1186/s13068-022-02211-0).

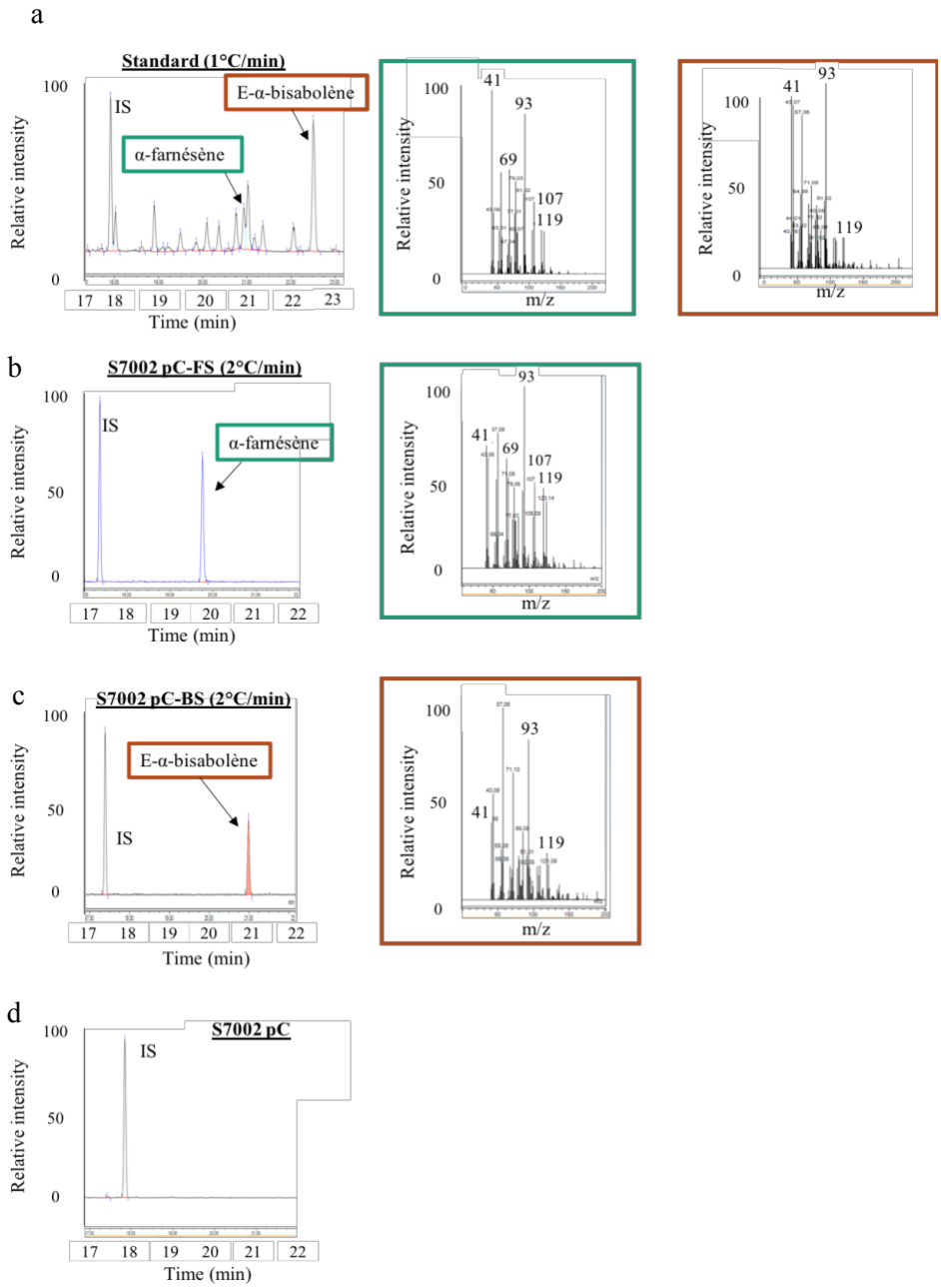


Figure S3B. GC–MS analyses of the dodecane overlay of cultures of the *Synechococcus* PCC 7002 strains harboring the plasmids pC or pCLS

Ion chromatograms (left panels) and corresponding mass spectra (right panels) of a S-(-)-limonene standard (upper panel) or dodecane samples (lower panel) of cultures of *Synechocystis* harboring either pC (negative control) or the pCLS (S-(-)-limonene production). Pinene (retention time =5.41) was used as the fixed-concentration (0.01 g/L) internal standard (IS) for quantification. The concentration of limonene was calculated using the standard curves that we employed during our recent analysis of limonene production by the other model cyanobacterium *Synechocystis* PCC 6803 (See Blanc-Garin *et al* 2022 Biotechnology for Biofuels and Bioproducts 15 https://doi.org/10.1186/s13068-022-02211-0).

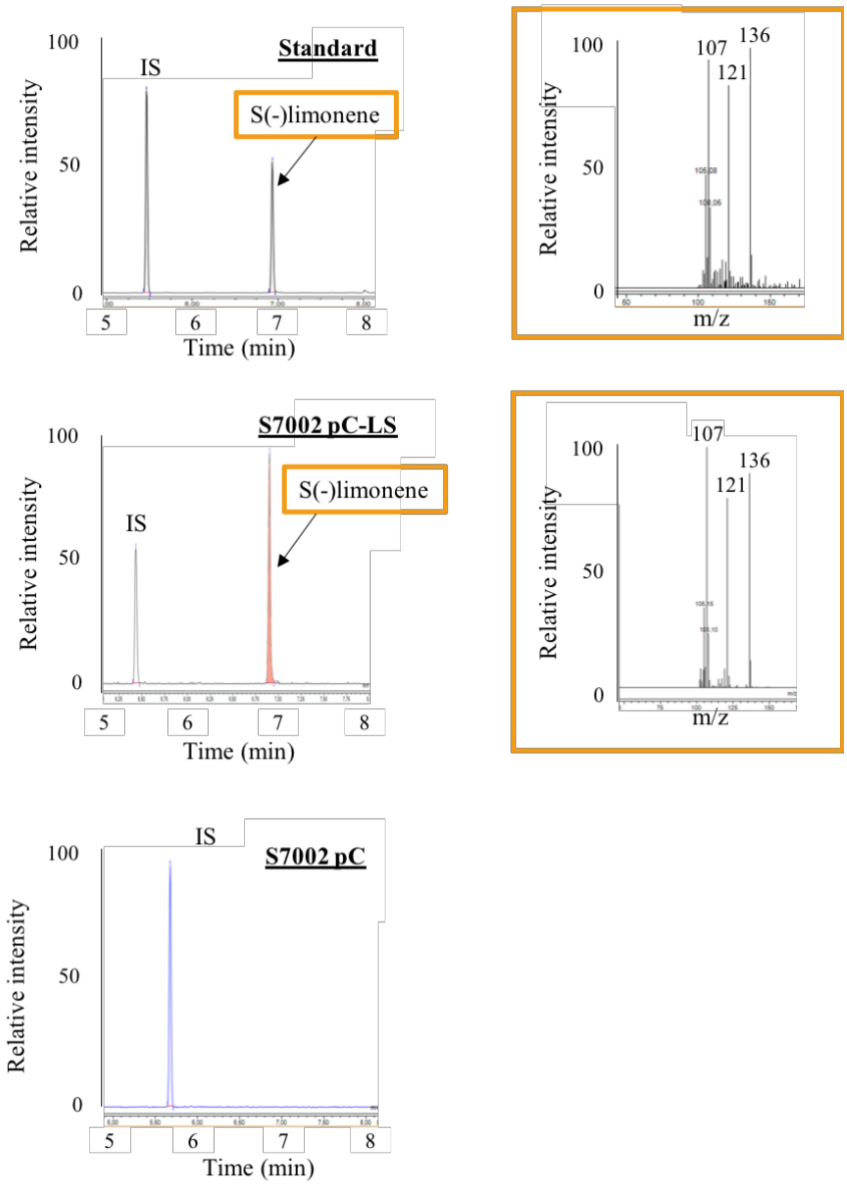


Figure S3C. GC–MS analyses of the dodecane overlay of cultures of the *Synechococcocus* PCC 7002 strains harboring the pC or pCPS plasmids

Ion chromatograms (left panels) and corresponding mass spectra (right panels) of an α-pinene standard (upper panel) or dodecane samples (lower panel) of cultures of *Synechococcus* PCC 7002 harboring either pC (negative control) or the pCPS (pinene production). S-(-)-limonene (retention time = 6.89 min) was used as the fixed-concentration (0.01 g/L) internal standard (IS) for quantification. The concentration of pinene was calculated using the standard curves that we employed during our recent analysis of pinene production by *Synechocystis* PCC 6803 (See Blanc-Garin *et al* 2022 Biotechnology for Biofuels and Bioproducts 15 https://doi.org/10.1186/s13068-022-02211-0).

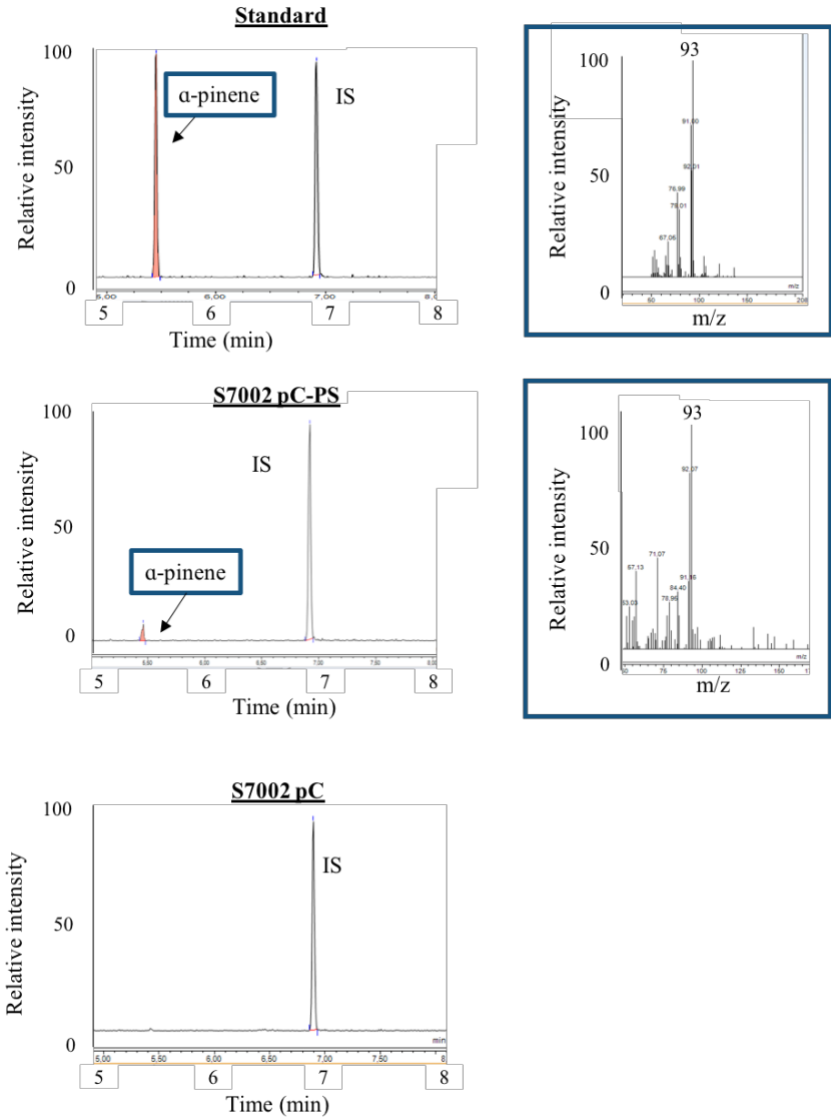


Figure S4A GC–MS analyses of the dodecane overlays of cultures of the *Synechoccocus* PCC 7942 strains harboring the plasmids pC, pCBS or pCFS

Ion chromatograms (left panels) and corresponding mass spectra (right panels) of farnesene-isomers standard containing bisabolene (**a**) or dodecane samples from cultures of *Synechococcus* PCC 7942 propagating pCFS (**b**, production of α-farnesene), pCBS (**c**, E-α-bisabolene production) or pC (**d**, negative control). β-caryophyllene (retention time = 17.3 min) was used as the fixed-concentration (0.01 g/L) internal standard (IS) for quantification. The concentrations of bisabolene and farnesene were calculated using the standard curves that we have reported during our recent analysis of the production of bisabolene and farnesene by the other model cyanobacterium *Synechocystis* PCC 6803 (See Blanc-Garin *et al* 2022 Biotechnology for Biofuels and Bioproducts 15 https://doi.org/10.1186/s13068-022-02211-0).

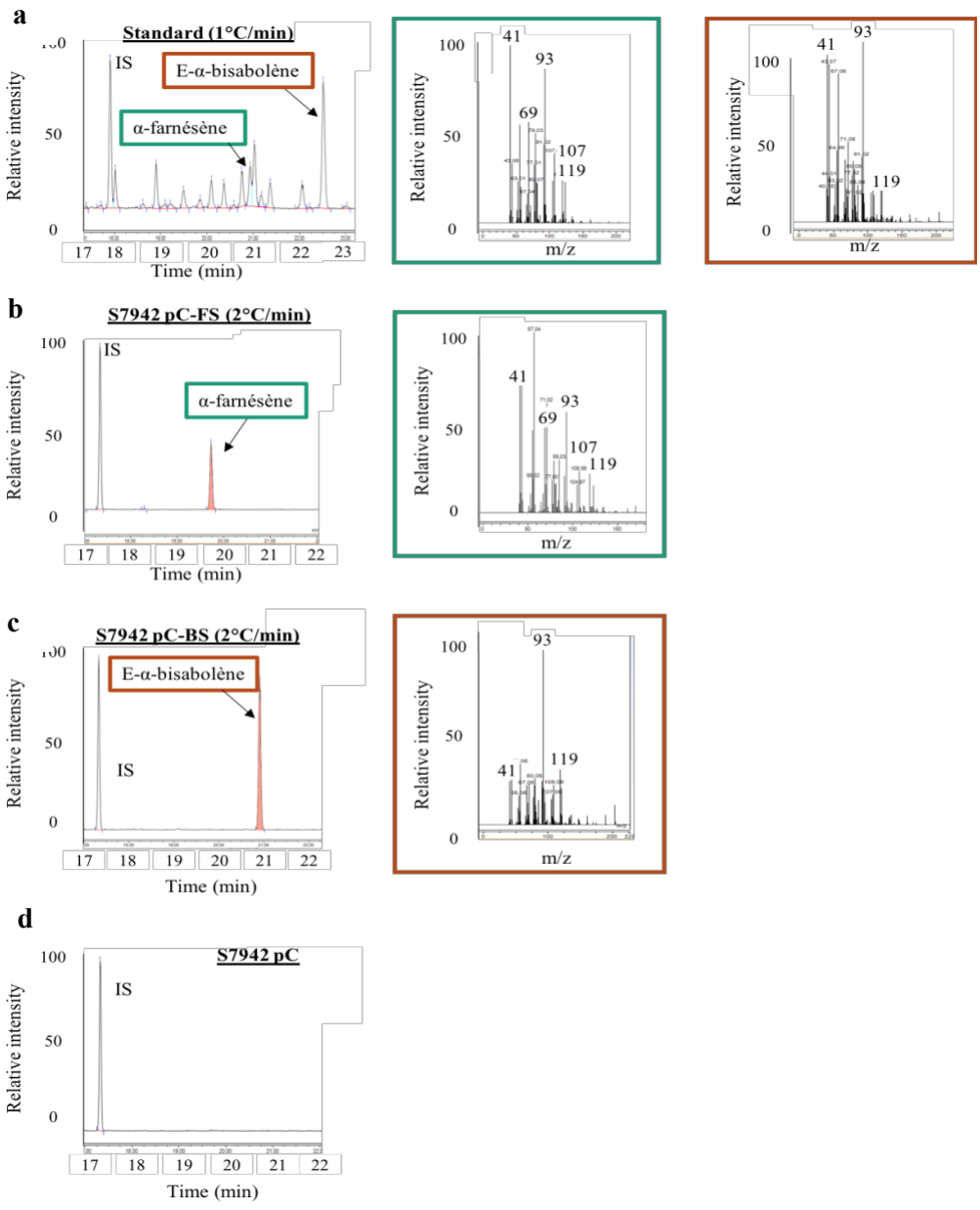
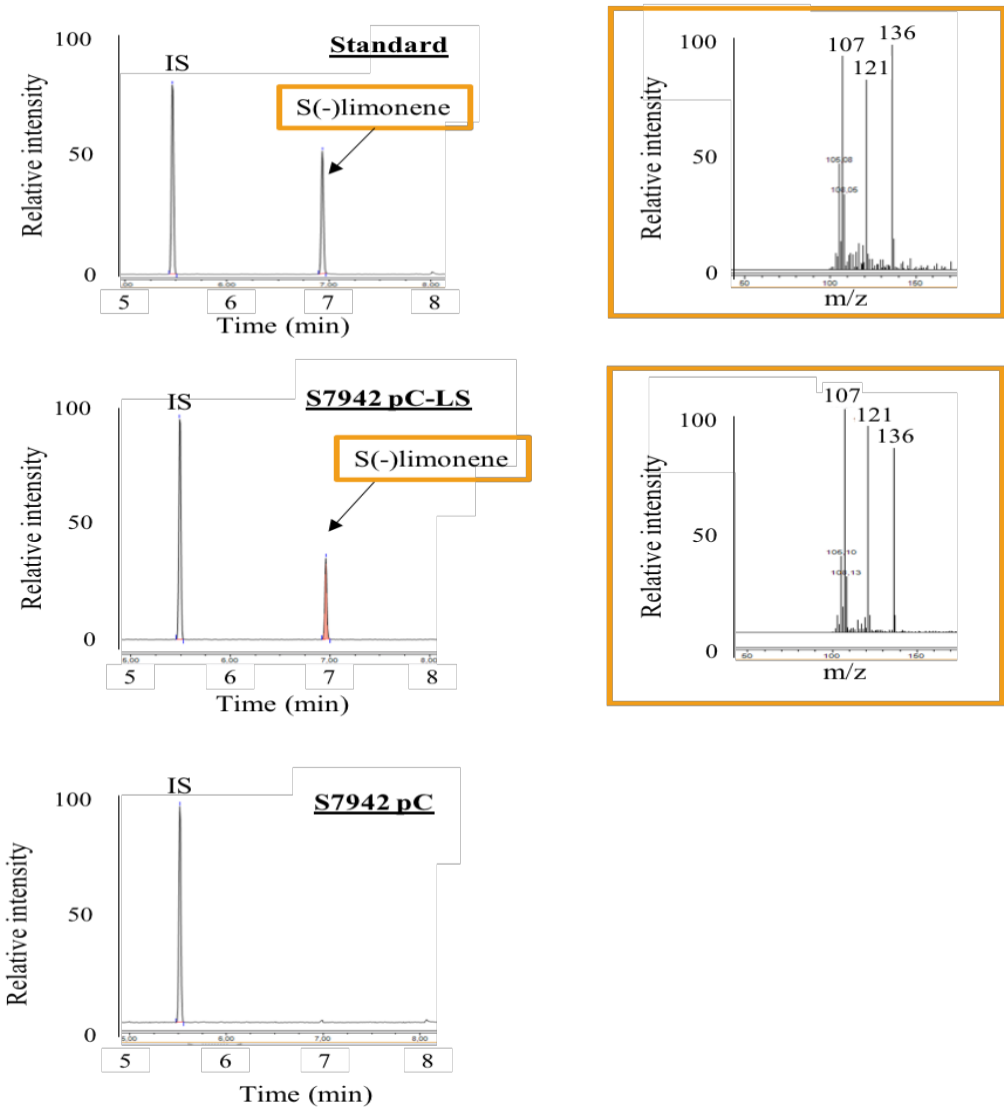


Figure S4B. GC–MS analyses of the dodecane overlay of cultures of the *Synechococcus* PCC 7942 strains harboring the pC or pCLS plasmids

Ion chromatograms (left panels) and corresponding mass spectra (right panels) of a S-(-)-limonene standard (upper panel) or dodecane samples (lower panel) of cultures of *Synechocccus* PCC 7942 harboring either pC (negative control) or the pCLS (S-(-)-limonene production). Pinene (retention time =5.44) was used as the fixed-concentration (0.01 g/L) internal standard (IS) for quantification. The concentration of limonene was calculated using the standard curves that we employed during our recent analysis of limonene production by the other model cyanobacterium *Synechocystis* PCC 6803 (See Blanc-Garin *et al* 2022 Biotechnology for Biofuels and Bioproducts 15 https://doi.org/10.1186/s13068-022-02211-0).



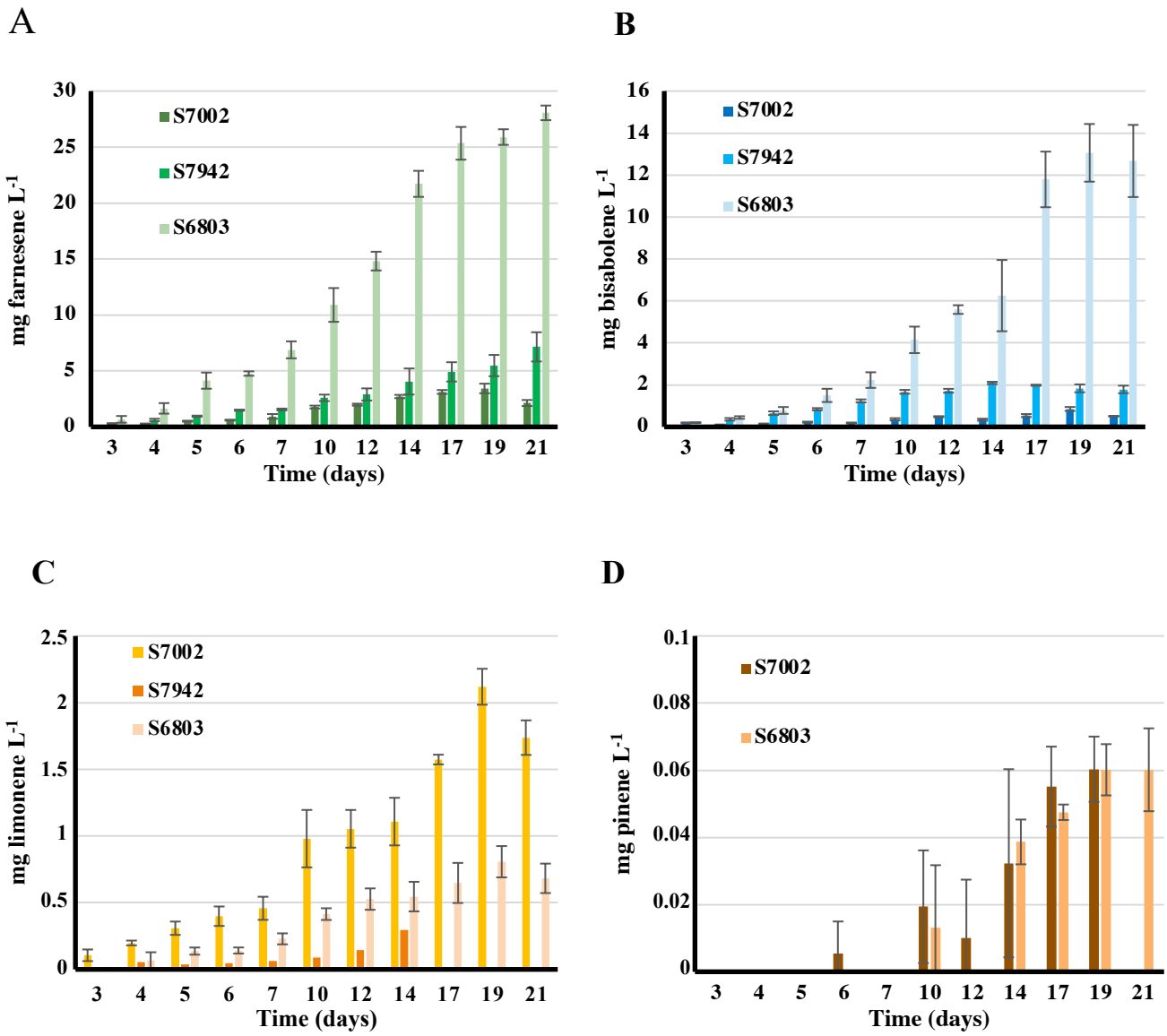


Figure S5. Comparison of the photosynthetic production of terpenes by the *Synechococcus* PCC 7002, *Synechococcus* PCC 7949 and *Synechocystis* PCC 6803 strains we have engineered in this study (S7002 and S7942), or a previous one (S6803)

Cells were grown under standard photoautotrophic conditions in the presence of a dodecane overlay (20% vol/vol) to assay the production of farnesene (A), Bisabolene (B), limonene (C) or Pinene (D) during 21 days. Error bars represent standard deviation from n≥ 2 biological replicates.

The terpenes producing strains of S6803 have been described in Blanc-Garin *et al* 2022 Biotechnology for Biofuels and Bioproducts 15 https://doi.org/10.1186/s13068-022-02211-0.

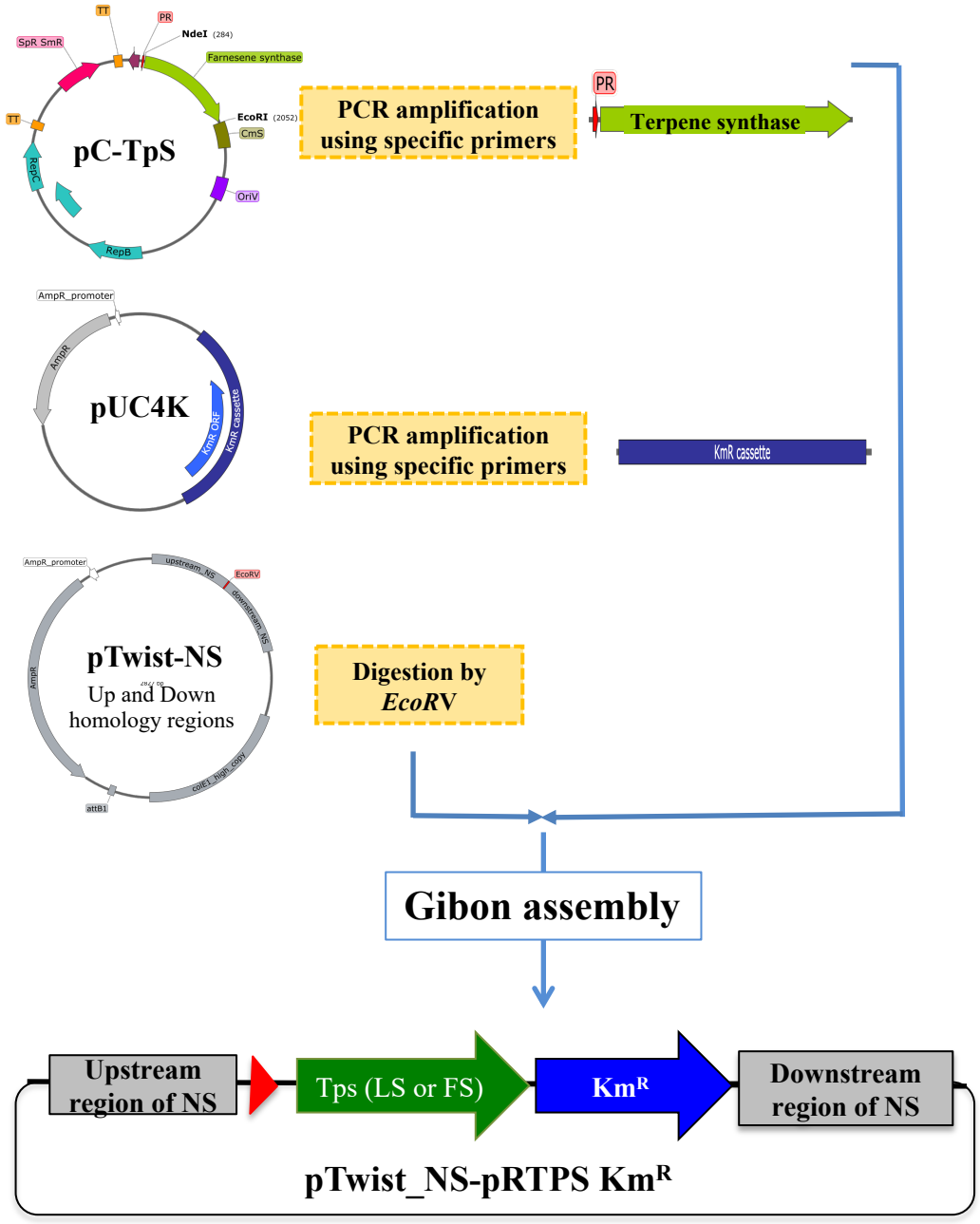


Figure S6. Construction of the pR-TPS-Km^R DNA cassette for targeted insertion of a terpene synthase genes (LS or FS) in the neutral chromosome sites of *Synechococcus* PCC 7002 (NS_{7002}) or *Synechococcus* PCC 7942 (NSI_{7942})

First, the ***pR-TPS*** gene (expression of the ***terpene synthase*** gene from the ***pR*** promoter) and the **Km^R** marker were PCR amplified from the plasmids pCTPS and pUC4K, respectively (Table S1). Second, the pTwist_NS plasmid was opened at its unique *Eco*RV restriction site flanked by the two chromosomal DNA regions surrounding the NS_{7002} or NSI_{7942} neutral sites (500 bp in length for NS_{7002} and 300 bp for NSI_{7942}). Third, all three DNA cassettes (***pR-TPS*** , **Km^R** and pTwist_NS) were assembled by Gibson® cloning. The resulting pTwist_NS_{7002}-TPS-Km^R and pTwist_NSI_{7942}-TPS-Km^R plasmids were transformed to S.7002 or S.7942 to insert the ***pR-TPS*_Km^R** cassette in the neutral chromosomic sites NS_{7002} or NSI_{7942}.

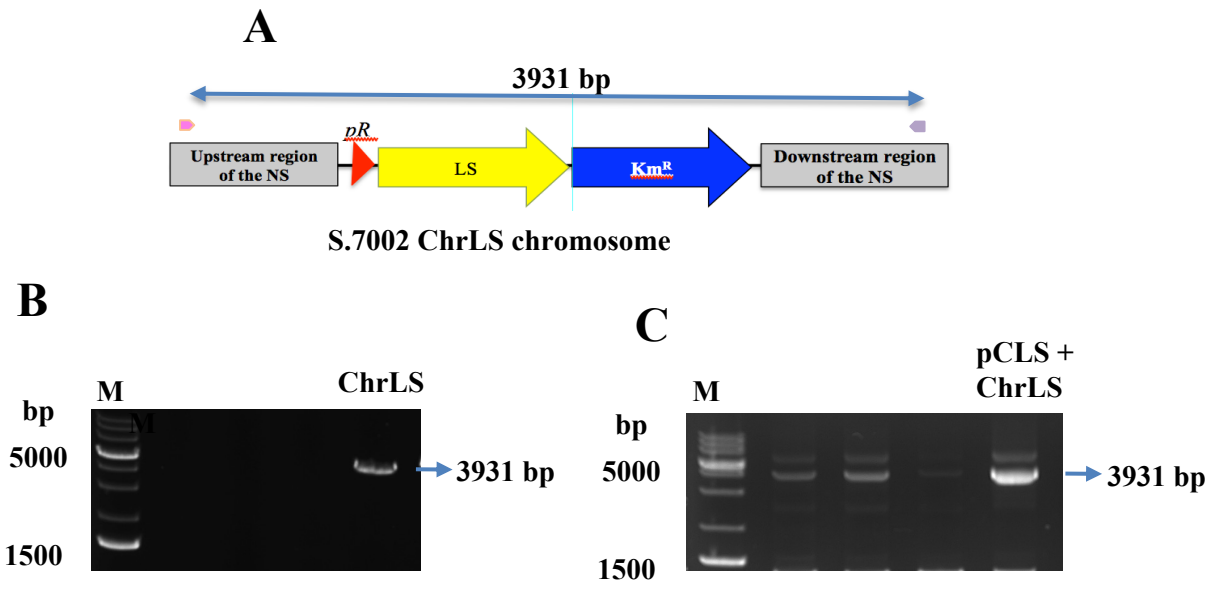


Figure S7. PCR verification of the presence of the pR-LS-Km^R cassette inserted inside the neutral chromosomal site (NS$_{7002}$) of *Synechococcus* PCC 7002.

A) Schematic representation of the pR-LS-Km^R DNA cassette integrated in the NS$_{7002}$ chromosomel site of S.7002. Genes are represented by wide shapes colored in yellow (limonene synthase gene, LS), grey (NS$_{7002}$ flanking regions), blue (Km^R marker) or red (*pR* promoter). PCR primers and the resulting 3931 bp DNA product are indicated by colored arrows.

B) Typical UV-light image of the agarose gel showing the 3931 bp PCR product of the ChrLS strain harboring the pR-LS-Km^R DNA cassette in the NS$_{7002}$ neutral chromosome site of otherwise WT cells of S.7002. Size marker (M) = Genruler 1 kb Plus DNA Ladder (Thermofisher).

C) Typical 3931 bp PCR product of the pCLS+ChrLS strain harboring the pR-LS-Km^R DNA cassette in the NS$_{7002}$ site of S.7002 cells propagating the pCLS plasmid. M = Genruler 1 kb Plus DNA Ladder (Thermofisher).

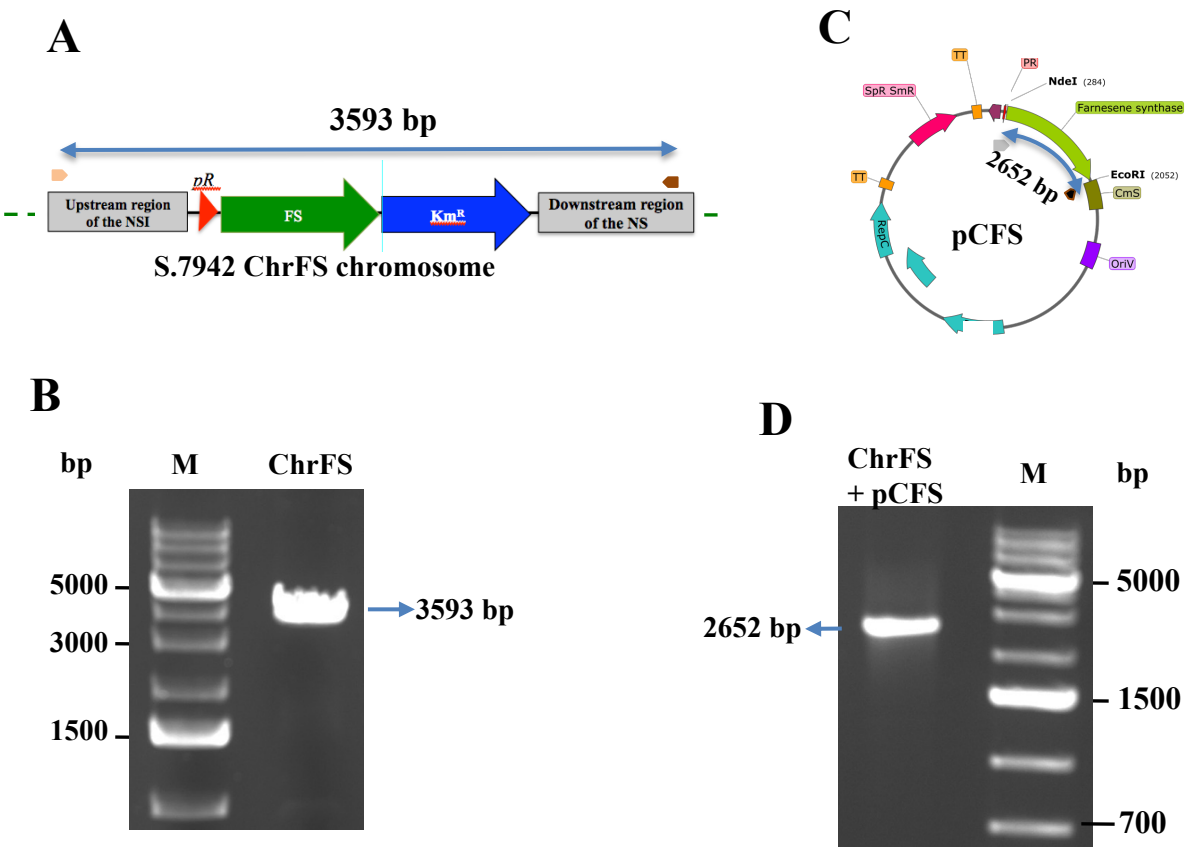


Figure S8. PCR verification of the presence of the farnesene synthase gene propagated by either or both the pCFS plasmid or the NSI_{7942} neutral chromosomal site of *Synechococcus* PCC 7942

A) Schematic representation of the pR-FS-Km^R DNA cassette integrated in the NSI_{7942} chromosome site of S.7942. Genes are represented by wide shapes colored in green (farnesene synthase gene, FS), grey (NSI_{7942} flanking regions), blue (Km^R marker) or red (*pR* promoter). PCR primers and the resulting 3593 bp DNA product are indicated by colored arrows.

B) Typical UV-light image of the agarose gel showing the 3593 bp PCR product of the ChrFS strain harboring the pR-FS-Km^R DNA cassette in the NSI_{7942} chromosome site of otherwise WT cells of S.7942. Size marker (M) = Genruler 1 kb Plus DNA Ladder (Thermofisher).

C) Schematic representation of the pCFS plasmid showing the *pR* promoter and FS gene as the small red triangle and green arrows, respectively. PCR primers and the corresponding 2652 bp DNA product are indicated by colored arrows.

D) Typical agarose gel showing the 2652 bp PCR product of the pR-FS gene of pCFS replicating in the ChrFS strain generating the ChrFS+pCFS strain. Size marker (M) = Genruler 1 kb Plus DNA Ladder (Thermofisher).

Table S1 – Characteristics of the bacterial strains and plasmids used in this study

Strain/Plasmid	Relevant features	Reference
***Escherichia coli* CM404**	*E. coli* strain harboring the self-transmissible plasmid pRK2013 enabling the conjugative transfer of RSF1010-derived pC plasmids into cyanobacteria	(Mermet-Bouvier and Chauvat, 1994)
***Escherichia coli* TOP10**	*E. coli* strain for cloning and conjugation	Invitrogen
***Escherichia coli* NEB 10-beta**	*E. coli* strain for cloning	New England Biolabs
***Synechococcus* PCC 7002**	Well studied, fast-growing unicellular and euryhaline cyanobacterium	Pasteur Institute (Paris, France)
***Synechococcus* PCC 7942**	Well studied unicellular freshwater cyanobacterium	Pasteur Institute (Paris, France)
Plasmid cloning vectors		
pTwist	Amp^R plasmid vector	Twist Biosciences
pUC4K	Source of the Km^R marker with no transcription terminator	Pharmacia
pC	RSF1010-derived plasmid vector (Sp^R/Sm^R, Cm^R) harboring the strong p_R promoter for constitutive gene expression in *E. coli* and cyanobacteria	(Veaudor et al., 2018)
Replicative plasmids for strong constitutive expression of terpene synthase genes in cyanobacteria		
pCLS	pC derived plasmid (Sp^R/Sm^R, Cm^S) harboring the *Mentha spicata* 4S-limonene synthase gene (LS) cloned between the *Nde*I and *EcoR*I restriction sites	(Chenebault *et al.*, 2020) & This study
pCPS	pC derivative (Sp^R/Sm^R, Cm^S) harboring the *Pinus taeda* pinene synthase gene (PS) cloned between *Nde*I and *EcoR*I	(Blanc-Garin *et al.*, 2022) & This study
pCBS	pC derivative (Sp^R/Sm^R, Cm^S) carrying the *Abies grandis* bisabolene synthase gene (BS) cloned between *Nde*I and *EcoR*I	(Blanc-Garin *et al.*, 2022) & This study
pCFS	pC derivative (Sp^R/Sm^R, Cm^S) carrying the *Picea abies* farnesene synthase gene (FS) cloned between *Nde*I and *EcoR*I	(Blanc-Garin *et al.*, 2022) & This study
Plasmids for gene cloning into the NS_{7002} neutral chromosomal site of *Synechococcus* PCC 7002		
pTwist_NS-S7002	pTwist harboring an *Eco*RV restriction site flanked by two 500 bp chromosomal DNA regions for cloning genes in the NS_{7002} neutral chromosome site of *Synechococcus* PCC 7002	This study (Twist Bioscience) and Figure S7
pTwist_NS-S7002p_RLSKm^R	pTwist_NS-S7002 harboring the Km^R DNA cassette expressing the *Mentha spicata* 4S-limonene synthase from the strong p_R promoter	This study; Fig.S7
Plasmids for gene cloning into the NS_{7942} neutral chromosomal site of *Synechococcus* PCC 7942		
pTwist_NSI-S7942	pTwist harboring an *Sma*I restriction site flanked by two 300 bp regions chromosomal DNA regions for cloning genes in the NSI_{7942} neutral chromosome site of *Synechococcus* PCC 7942	This study (Twist Bioscience) Figure S7
pTwist_NSI-S7942-p_RFSKm^R	pTwist_NS-S7942 harboring the Km^R DNA cassette expressing the *Picea abies* farnesene synthase gene (FS) from the strong p_R promoter	This study; Figure S8

References:
- Mermet-Bouvier, P.; Chauvat, F.. *Curr. Microbiol.* **1994**, *28*, 145–148, doi:10.1007/BF01571055.

- Chenebault, C. *et al*. *Front. Microbiol.* **2020**, *11*, 586–601, doi:10.3389/fmicb.2020.586601.

- Blanc-Garin, V.; Chenebault, C. et al. .. *Biotechnol. Biofuels* **2022**, *15*, doi:10.1186/s13068-022-02211-0.

Table S2 – Characteristics of the primers used in this study

Name	Sequence (5'-3')	Purpose
pC and pC derivatives		
pC_Fw	TCATAAATTGCTTTAAGGCG	Forward (Fw) and reverse (Rv) primers for PCR amplification and DNA sequencing of the terpene synthase genes cloned in pC
pC_Rv	GTGTAACAAGGGTGAACAC	
pTwist plasmids		
M13_Fw	GTAAAACGACGGCCAGT	DNA sequencing of inserts cloned in pTwist
M13_Rv	CAGGAAACAGCTATGAC	
Pinene synthase encoding gene		
PS_Fw1	GCGAGAAGTTGCTAGAATTGGC	Forward and reverse primers for DNA sequencing of PS genes
PS_Rv1	TAACCGCAAAATGGCGCAAA	
PS_Rv2	ACCAACGAGACAGAGACTGC	
Limonene synthase encoding gene		
LS_Rv3	GATAGGCCCATGCGTTGAAGAT	Forward and reverse primers for DNA sequencing of LS genes
LS_Fw3	TGTCCGATTATAATGCCTCCG	
Bisabolene synthase encoding gene		
BS_Fw2	TGAGACCGACCAAGATAGC	Forward and reverse primers for DNA sequencing of BS genes
BS_Fw3	TAGGCATTGGAACGAGCG	
BS_Fw4	TATGACACCTACGGTACC	
BS_Rv5	CAAGAACTCAAGGCACTTC	
Construction of the NS_{7002}::p*R*-LS-Km^R and NSI_{7942}::p*R*-FS-Km^R DNA cassettes by GIBSON assembly		
Pr-fwd-S7942	gacgagcagggactcgacccTTGACTATTTTACCTCTGGC	Forward primer for PCR amplification of the *pR*-FS DNA cassette of the pCFS plasmid. It overlaps the upstream region of the NSI_{7942} chromosomal site (in 5') and *pR* (in 3')
Pr-fwd-S7002	acttaaaaactgagtaagatTTGACTATTTTACCTCTGGC	Forward primer for PCR amplification of the *pR*-LS and *pR*-FS DNA cassettes of the pCLS and pCFS plasmids. It overlaps the upstream region of the NS_{7002} chromosomal site (in 5') and *pR* (in 3')
Km-rev-S7002	aaaacgagataaaataggatGACCTGCAGGGGGGGGGG	Reverse primer for PCR amplification of the Km^R gene of pUC4K. It overlaps the downstream region of NS_{7002} (in 5') and the end of Km^R (in 3')

Pr-FS-Rv	cctgcaggtcTTACATGGGAACGGGTTC	Reverse primer for PCR amplification of the *pR*-FS cassette of pCFS. It overlaps the end of the FS gene (in 3') and the beginning of the Km^R marker (in 5')
Pr-LS-Rv	cctgcaggtcTTAAGCAAAGGGCTCAAAC	Reverse primer for PCR amplification of the *pR*-LS cassette of pCLS. It overlaps the end of the LS gene (in 3') and the beginning of Km^R (in 5')
KM-FS-Fwd	tcccatgtaaGACCTGCAGGGGGGGGG	Forward primer for PCR amplification of the Km^R gene of pUC4K. It overlaps the end of FS (in 3') and the beginning of Km^R (in 5')
KM-LS-Fwd	ctttgcttaaGACCTGCAGGGGGGGGG	Forward primer for PCR amplification of the Km^R gene of pUC4K. It overlaps the end of LS (in 3') and the beginning of Km^R (in 5')
KM-rev-S7942	ttcctgctccagaagccccGACCTGCAGGGGGGGGG	Reverse primer for PCR amplification of the Km^R gene of pUC4K. It overlaps the downstream region of NSI neutral site (in 5') and the end of Km^R (in 3')
Plasmids and *Synechococcus* PCC 7002 and *Synechococcus* PCC 7942 strains harboring the DNA cassettes		
NS_S7002_Fw	ATTGGGGAATAGTGCGAC	PCR amplification of the *pR*-LS-Km^R and *pR*-FS-Km^R DNA cassette inserted into the NS_{7002} chromosomal site of S.7002, and analysis of chromosome segregation
NS_S7002_Rv	ATGTGCTTTCAGCAAAAGTT	
NS7942_Fwd	CACAGACATCTAGATAGTCCTCAA	PCR amplification and DNA sequencing of the FS expression cassette (*pR*-FS-Km^R) cloned into the NSI_{7942} chromosomal site of S.7942, and analysis of chromosome segregation.
NS7942_Rv	CTGAAGCGGACATTGCTA	