Microbial Cell Factories

Additional file 1

Green light-induced production of isobutanol and 3-methyl-1-butanol by metabolically engineered cyanobacteria

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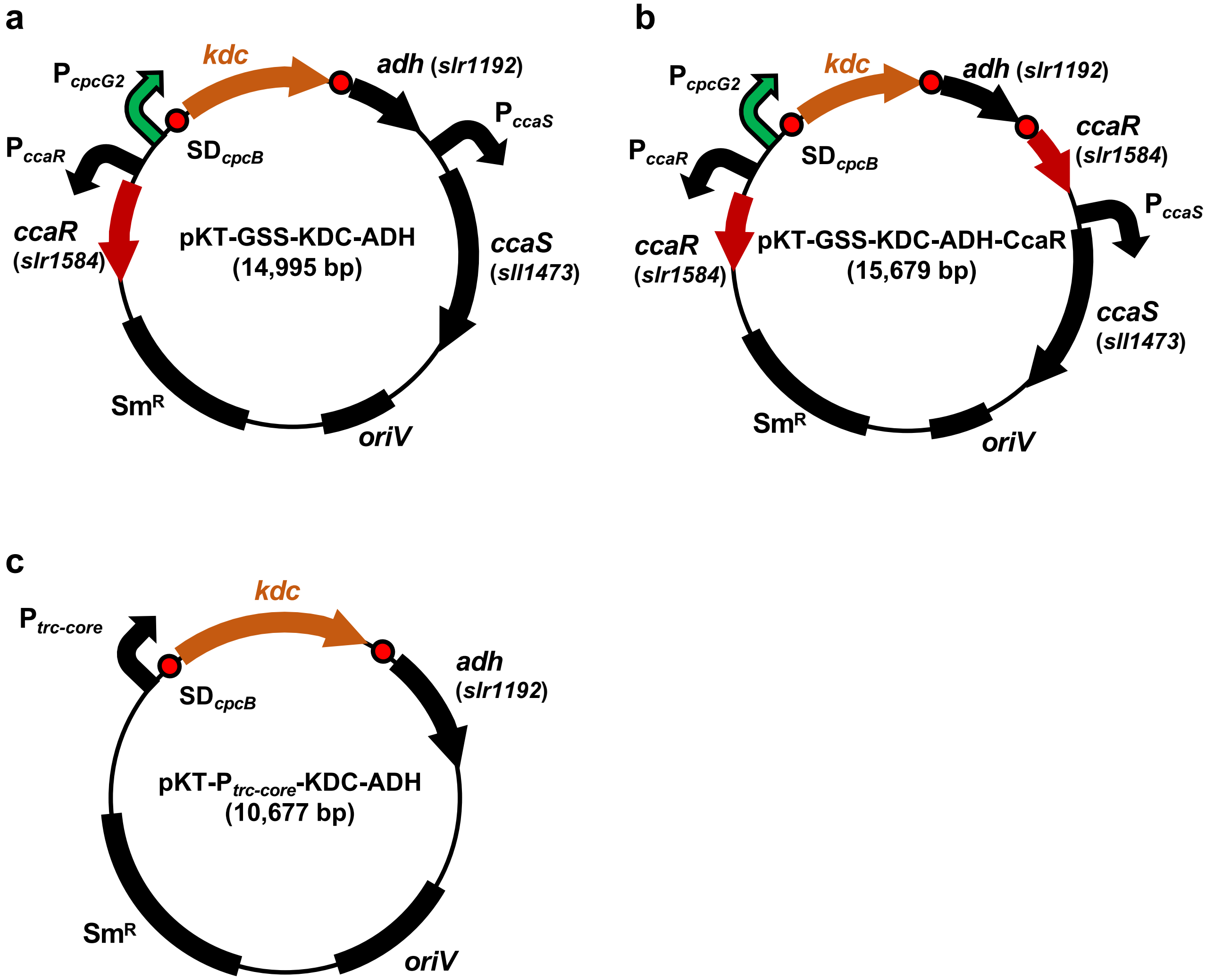

Fig. S1 Design of vectors used in this study. **(a)** pKT-GSS-KDC-ADH for the application of CcaS/CcaR system to produce alcohol in cyanobacteria. pKT-GSS-KDC-ADH was constructed by inserting *kdc* and *adh* into the downstream of P_{cpcG2} on pKT-GSS. **(b)** pKT-GSS-KDC-ADH-CcaR to tune the CcaS/CcaR system up for the enhancement of alcohol production. pKT-GSS-KDC-ADH-CcaR was constructed by inserting the structural genes of *kdc*, *adh,* and *ccaR* into the downstream of P_{cpcG2} on pKT-GSS. **(c)** pKT-$P_{trc\text{-}core}$-KDC-ADH for the constitutive activation of alcohol production in cyanobacteria. The *ori*V origin is replicated in *Synechocystis* sp. PCC 6803. Gene abbreviations are as follows: P_{ccaS}, *ccaS* promoter; P_{ccaR}, *ccaR* promoter; P_{cpcG2}, *cpcG2* promoter; *kdc*, keto-acid decarboxylase; *adh*, alcohol dehydrogenase; Sm^R, streptomycin-resistance gene; SD_{cpcB}, Shine Dalgarno-like sequence of *cpcB* derived from *Synechococcus* sp. PCC 7002.

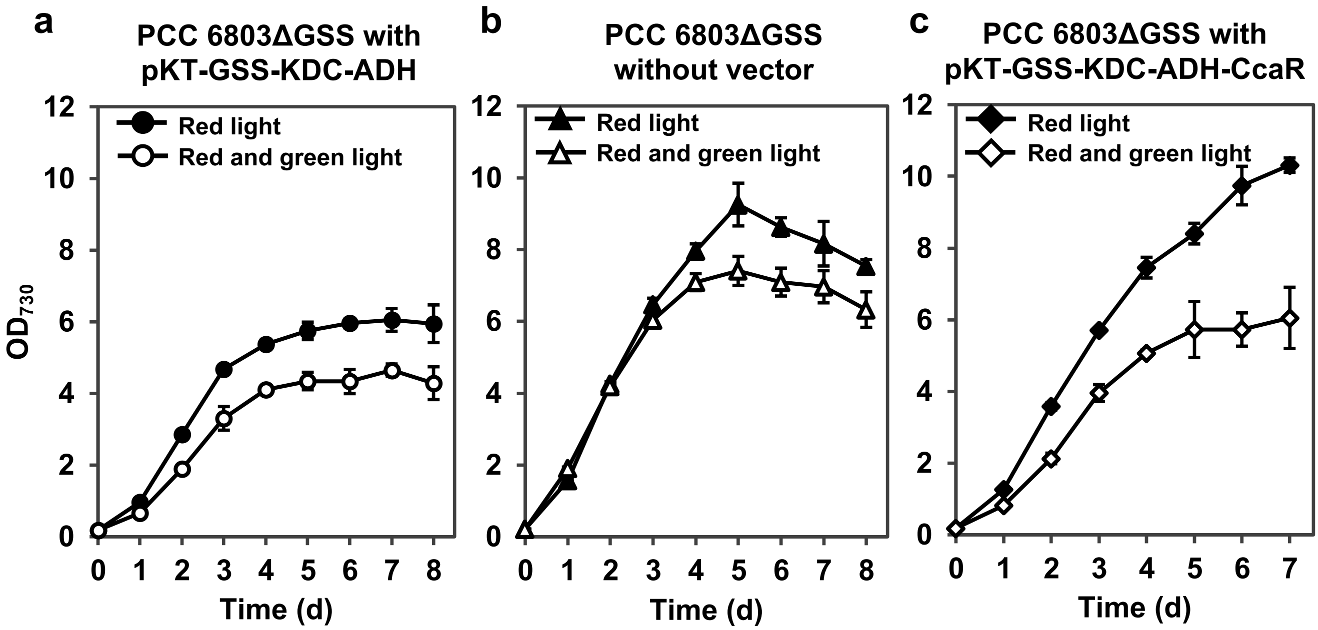

Fig. S2 Growth curves of each strain used in this study. Growth curves of **(a)** the PCC 6803ΔGSS strain with pKT-GSS-KDC-ADH, **(b)** the PCC 6803ΔGSS strain, and **(c)** the PCC 6803ΔGSS strain with pKT-GSS-KDC-ADH-CcaR. All strains were cultivated under the red light illumination alone (black markers) and under the red and green light illumination (white markers). All analyses were performed in triplicate. Error bars represent the standard deviation (SD) calculated from triplicate experiments using one clone.

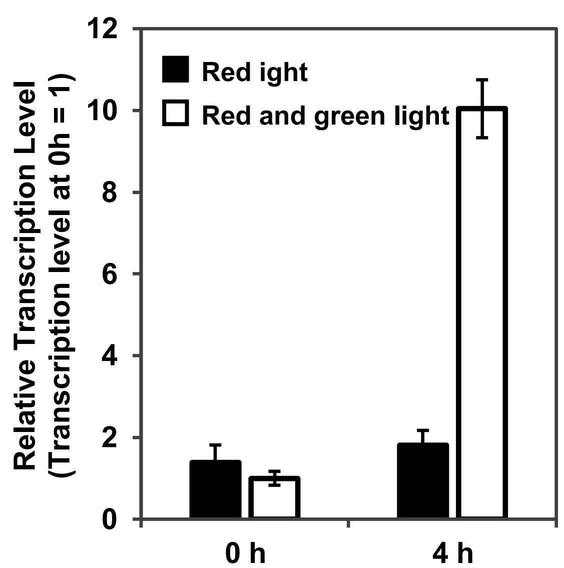

Fig. S3 Transcriptional analysis of *kdc* gene in the *Synechocystis* sp. PCC 6803ΔGSS strain. The relative gene transcription levels of *kdc* in the transformants of *Synechocystis* sp. PCC 6803ΔGSS integrated with pKT-GSS-KDC-ADH cultivated under the red light illumination alone (black bar) and under the red and green light illumination (white bar) are shown. All analyses were performed in triplicate. Error bars represent the SD calculated from triplicate experiments using one clone.

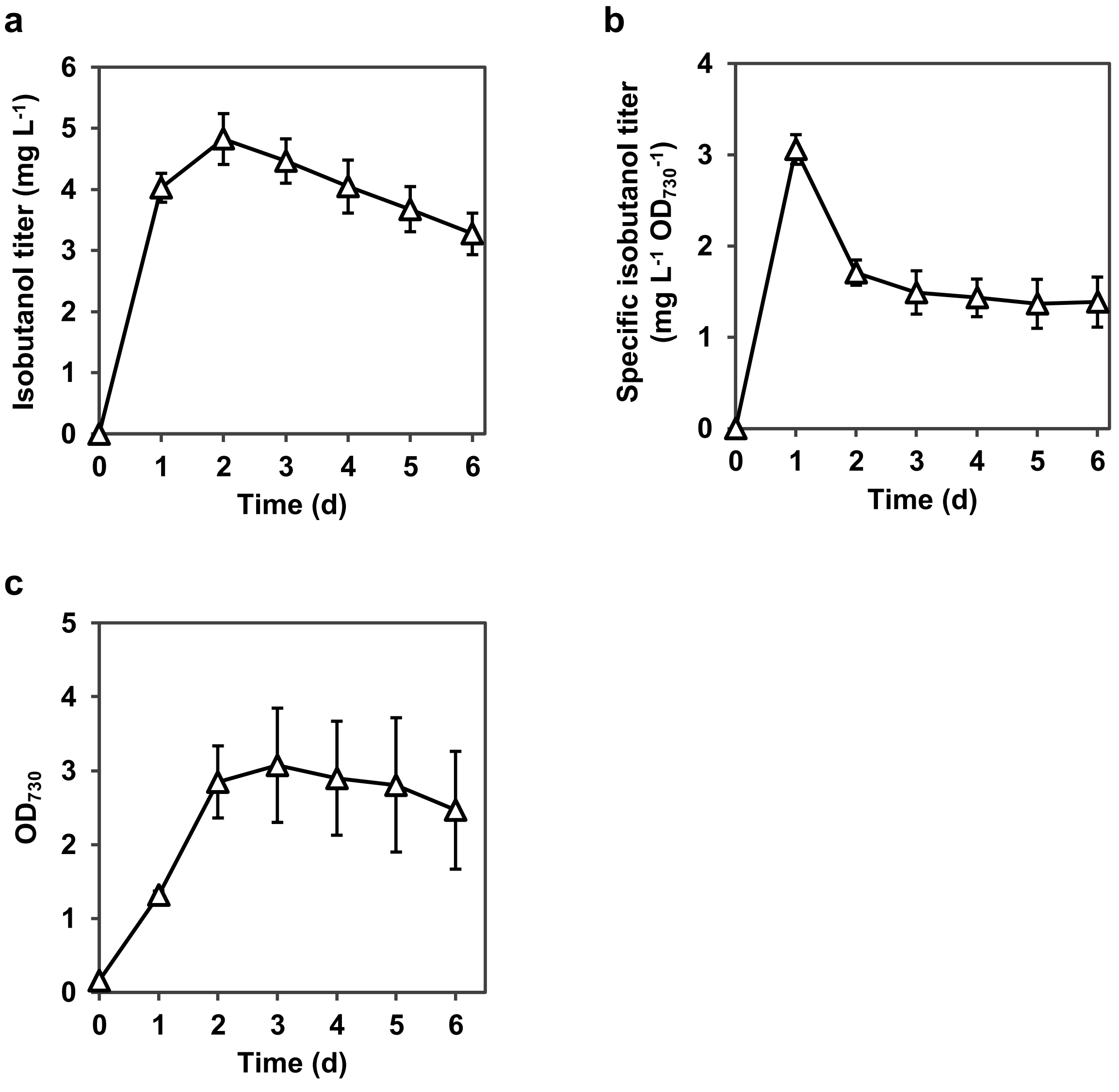

Fig. S4 Evaluation of alcohol production utilizing the $P_{trc\text{-}core}$ system in *Synechocystis* sp, PCC 6803ΔGSS. **(a)** Production titer of isobutanol in the culture supernatant of *Synechocystis* sp. PCC 6803ΔGSS transformed with pKT-$P_{trc\text{-}core}$-KDC-ADH cultivated under the red and green light illumination. **(b)** Specific titer of isobutanol under the red and green light illumination. **(c)** Growth curve of the transformant cultivated under the red and green light illumination. All analyses were performed in triplicate. Error bars represent the SD calculated from triplicate experiments using one clone.

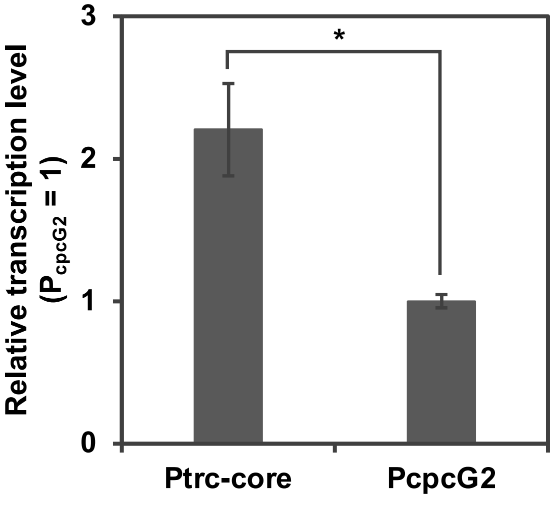

Fig. S5 Comparison of transcription level of *kdc* gene between the $P_{trc\text{-}core}$ and the P_{cpcG2}. Transcription analysis was conducted after 24 h of cultivation of the PCC 6803ΔGSS strain transformed with pKT-$P_{trc\text{-}core}$-KDC-ADH or pKT-GSS-KDC-ADH under red and green light illumination. All analyses were performed in triplicate. Error bars represent the SD calculated from triplicate experiments using one clone. Statistical analysis was performed using Student's *t*-test: * $p < 0.05$.

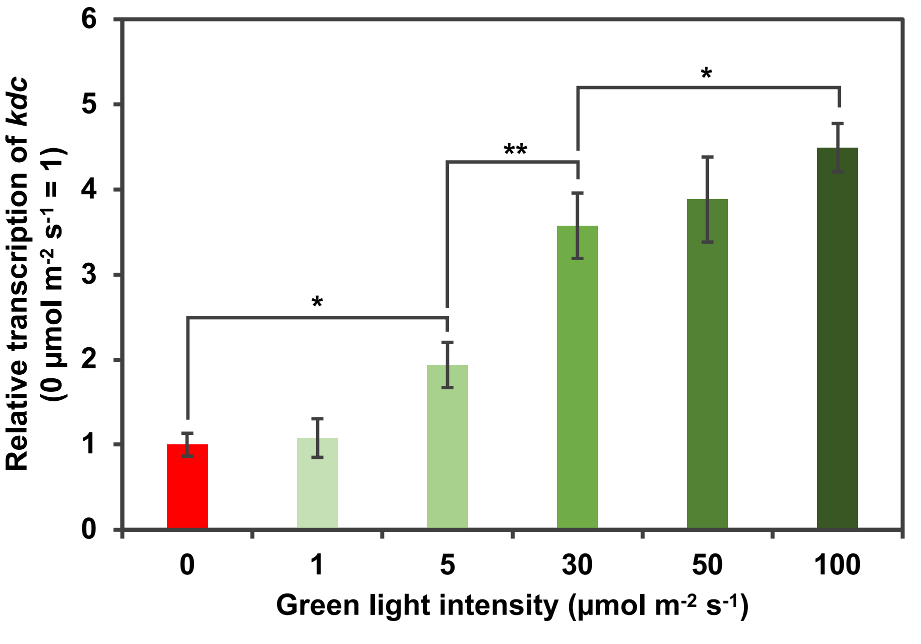

Fig. S6 Comparison of transcription level of *kdc* gene from the P_{cpcG2} under the various intensities of green light. Transcription level of *kdc* in the PCC 6803ΔGSS strain transformed with pKT-GSS-KDC-ADH under green light illumination (0 µmol m^{-2} s^{-1}, 1 µmol m^{-2} s^{-1}, 5 µmol m^{-2} s^{-1}, 30 µmol m^{-2} s^{-1}, 50 µmol m^{-2} s^{-1}, or 100 µmol m^{-2} s^{-1}) with red light illumination (30 µmol m^{-2} s^{-1}) was shown. All analyses were performed in triplicate. Error bars represent the SD calculated from triplicate experiments using one clone. Statistical analysis was performed using one-way analysis of variance (ANOVA) with Tukey's multiple comparison test: * $p < 0.05$, ** $p < 0.005$.

Table S1 Comparison of the isobutanol production titer in cyanobacteria expressing keto-acid decarboxylase (*kdc*) and alcohol dehydrogenase (*adh*) in this study with that in previous studies.

Strain	Promoter (Inducer)	Genes	Titer	Productivity*	Production time	Ref.
PCC 7942	P_{trc} (IPTG)	*kdc*, *yqhD*	50 mg L^{-1}	17 mg L^{-1} d^{-1}	3 d	[3]
PCC 6803	P_{tac} (No inducer)	*kdc*, *adhA*	90 mg L^{-1}	15 mg L^{-1} d^{-1}	6 d	[26]
PCC 6803	$P_{trc\text{-}core}$ (No inducer)	*kdc* (S286T)	911 mg L^{-1}	20 mg L^{-1} d^{-1}	46 d	[28]
PCC 6803ΔGSS	P_{cpcG2} (Green light)	*kdc*, *slr1192*	238 mg L^{-1}	48 mg L^{-1} d^{-1}	5 d	This study

* The productivity was calculated as the average production level per day by dividing the titer by the number of days required for production.

Table S2 Comparison of the isobutanol production in this study with the highest titer and productivity achieved in previous studies.

Strain	Promoter (Inducer)	Genes	Titer	Productivity*	Production time	Ref.
PCC 7942	P_{trc}, P_{LlacO1} (IPTG)	*kdc*, *yqhD*, *alsS*, *ilvC*, *ilvD*	450 mg L^{-1}	75 mg L^{-1} day^{-1}	6 d	[3]
PCC 7942Δ*glgC*	P_{trc}, P_{LlacO1} (IPTG)	*kdc*, *yqhD*, *alsS*, *ilvC*, *ilvD*	550 mg L^{-1}	69 mg L^{-1} day^{-1}	8 d	[24]
PCC 7942	P_{trc}, P_{LlacO1} (IPTG)	*kdc*, *adhA*, *alsS*, *ilvC*, *ilvD*	637 mg L^{-1}	32 mg L^{-1} day^{-1}	20 d	[25]
PCC 6803	$P_{trc\text{-}core}$ (No inducer)	*kdc* (S286T)	911 mg L^{-1}	20 mg L^{-1} day^{-1}	46 d	[28]
PCC 6803ΔGSS	P_{cpcG2} (Green light)	*kdc*, *slr1192*	238 mg L^{-1}	48 mg L^{-1} day^{-1}	5 d	This study

* The productivity was calculated as the average production level per day by dividing the titer by the number of days required for production.

Table S3 Comparison of the 3-methyl-1-butanol titer in cyanobacteria obtained in this study with that in previous studies.

Strain	Promoter (Inducer)	Genes	Titer	Productivity*	Production time	Ref.
PCC 7942Δ*glgC*	P_{trc}, P_{LlacO1} (IPTG)	*kdc*, *yqhD*, *alsS*, *ilvC*, *ilvD*	70 mg L^{-1}	10 mg L^{-1} d^{-1}	7 d	[20]
PCC 6803	$P_{trc\text{-}core}$ (No inducer)	*kdc* (S286T)	225 mg L^{-1}	5 mg L^{-1} d^{-1}	46 d	[23]
PCC 6803ΔGSS	P_{cpcG2} (Green light)	*kdc*, *slr1192*	75 mg L^{-1}	15 mg L^{-1} d^{-1}	5 d	This study

* The productivity was calculated as the average production level per day by dividing the titer by the number of days required for production.

Table S4 Sequences of the vector inserts used in this study.

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pKT-P$_{trc\text{-}core}$-KDC-ADH

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*The regions encoding *ccaR* (Turquoise), P_{cpcG2} (Green), Shine-Dalgarno-like sequence derived from *cpcB* from *Synechococcus* sp. PCC 7002 (Gray), *kdc* (Yellow), *adh* (Pink) and *ccaS* (Red), $P_{trc\text{-}core}$ (Dark red) were shown.

Table S5 Primer sequences used in this study.

Primer No.	Sequence (5' to 3')
P01	GTTTAATTACTAACTAGATCTTATAAGTAGGAGATAAAAACATGTATACGGTTGGAGATTATCTGC
P02	TATCTCCTACTTATATTAGGATTTGTTCTGTTCTGCGAAGAG
P03	CCTAATATAAGTAGGAGATAAAAACATGATTAAAGCCTACGCTGCCC
P04	TTACCGTCAAAAAAATCTAGACTAATTTTTACTATGGCTGAGCACTACCC
P05	GTTTAATTACTAACTAGATCTTATAAGTAGGAGATAAAAACATG
P06	CTACTTATACTAATTTTTACTATGGCTGAGCACTACCC
P07	GTAAAAATTAGTATAAGTAGGAGATAAAAACATGAGAATTCTTTTAGTG
P08	TTACCGTCAAAAAAATCTAGACTAGTTTTTCCCTTGGCACAAAG
P09	AATTCTGCAGGAGCTGTTGACAATTAATCATCCGGCTCGTATAATGTGTGGAAGATCTTATAAGTAGGAGATAAAAACATGTATACGG
P10	AATTCTGCAGGCGAAAAAACCCCGCCGAAGCGGGGTTTTTTGCGTCTAGACTAATTTTTACTATGGCTGAGCAC
P11	GGTTGGTAACGCCAATGAAC
P12	ACCAAAGGTCGTCAAGAACG
P13	GCTAATACCCAATGTGCCGA
P14	CACTGCTGCCTCCCGTAG