Transcriptional terminators allow leak-free chromosomal integration of genetic constructs in cyanobacteria

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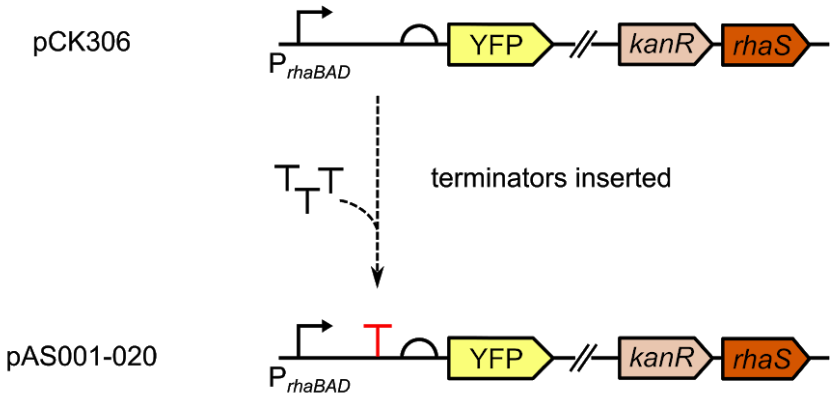
Supplementary Results

A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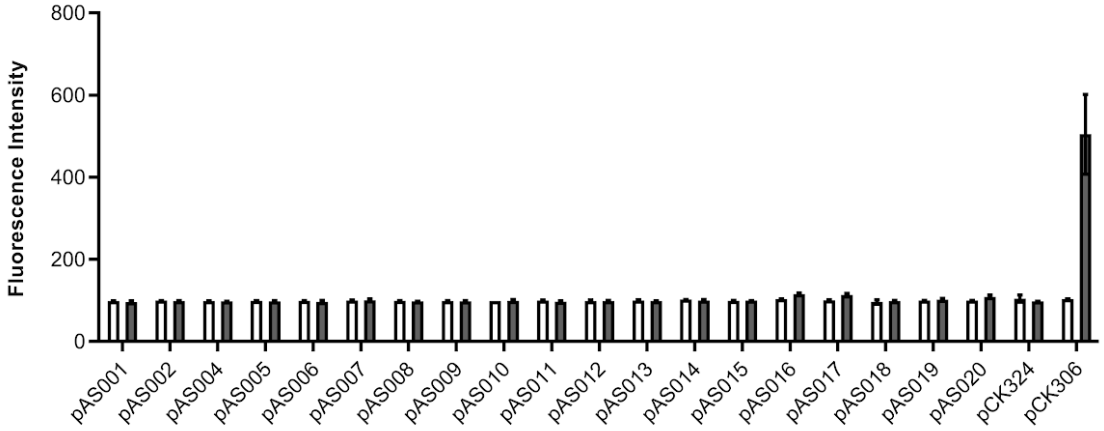
YFP
kanR
rhaS
terminators inserted
pAS001-020
P rhaBAD
YFP
kanR
rhaS

B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pAS005
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pAS010
pAS011
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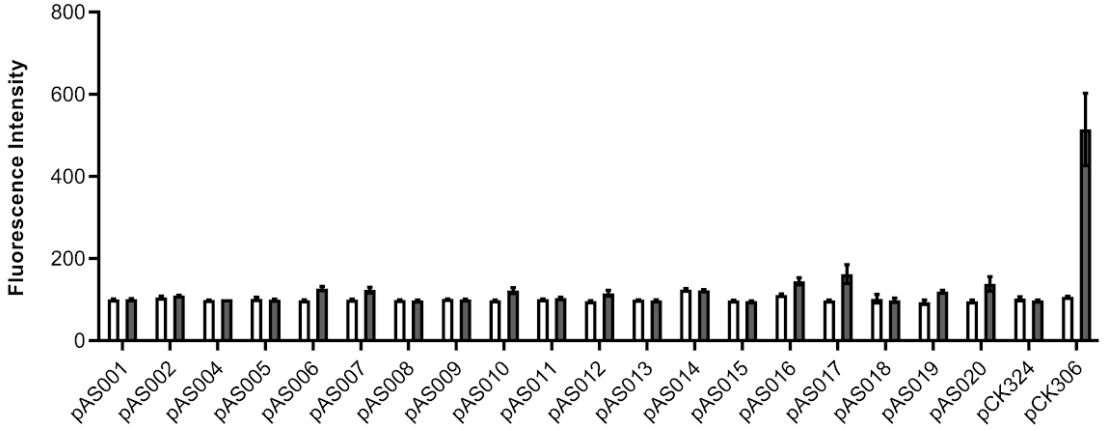
pAS014
pAS015
pAS016
pAS017
pAS018
pAS019
pAS020
pCK324
pCK306

C


Fluorescence Intensity
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pAS001
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pAS011
pAS012
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pAS015
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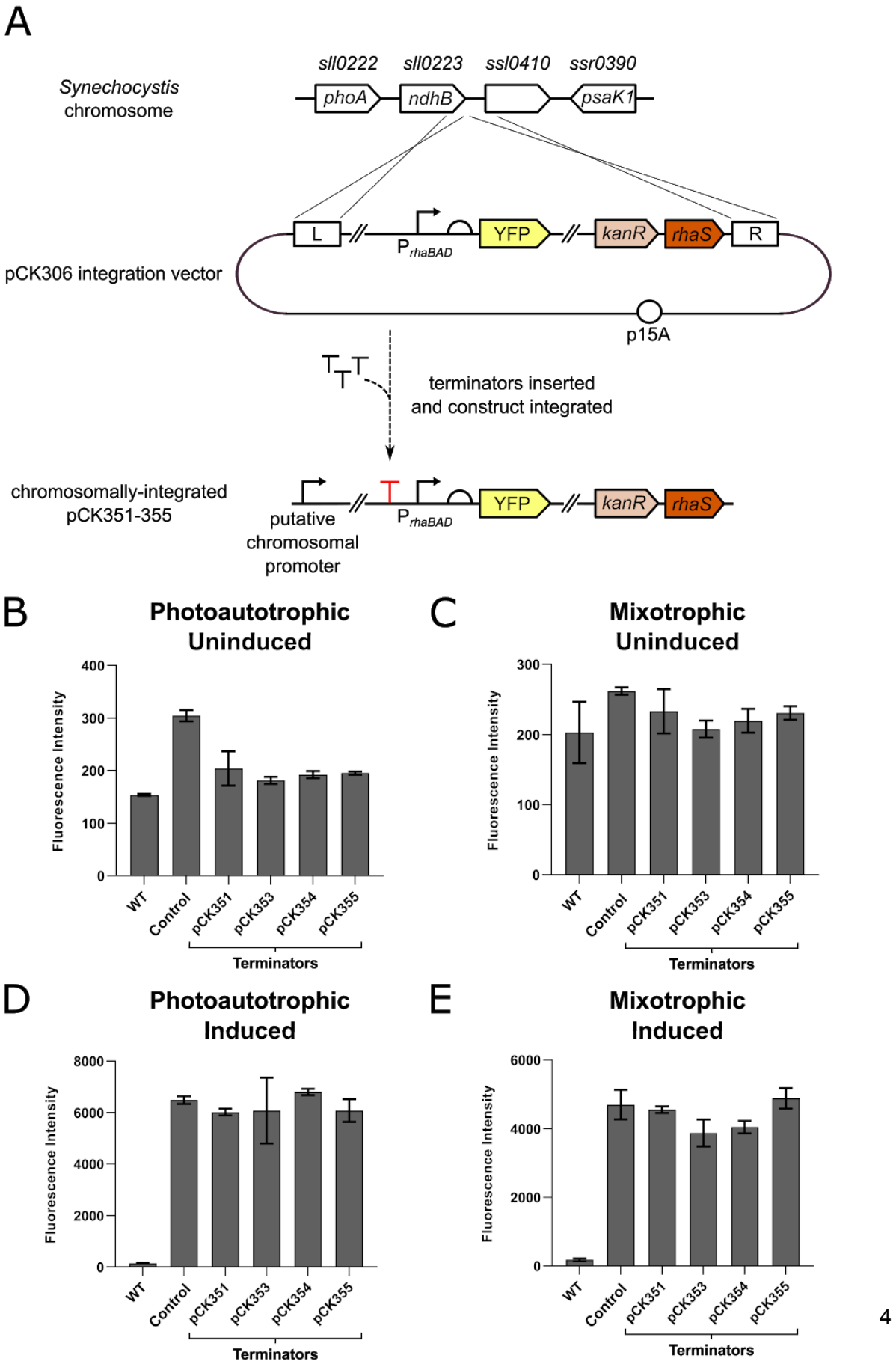
pAS017
pAS018
pAS019
pAS020
pCK324
pCK306

Figure S1. Efficiency of terminators in *E. coli* strains DH5α and MG1655. *E. coli* strains (A) DH10β and (B) MG1655 containing plasmids pAS001-002, pAS004-020 (terminator between *rhaBAD* promoter and RBS of YFP-encoding gene) were cultured in LB media supplemented with kanamycin and 0 mg/ml L-rhamnose (white bars) or 0.6 mg/ml L-rhamnose (black bars). Cells containing pCK324 (lacking *rhaBAD* promoter and therefore no YFP) and pCK306 (the parental vector with no terminator and therefore fully inducible with L-rhamnose) were used as controls. The fluorescence intensity of 10,000 cells (arbitrary units) from each culture was measured by flow cytometry after 6 h. Error bars shown are the standard deviation of the mean for three independent biological replicates.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sll0222
sll0223
ssl0410
ssr0390
Synechocystis chromosome
phoA
ndhB
psaK1
L
P rhaBAD
YFP
kanR
rhaS
R
pCK306 integration vector
p15A
terminators inserted and construct integrated
chromosomally-integrated pCK351-355
putative chromosomal promoter
B
Photoautotrophic Uninduced
C
Mixotrophic Uninduced
D
Photoautotrophic Induced
E
Mixotrophic Induced
Fluorescence Intensity
WT
Control
pCK351
pCK353
pCK354
pCK355
Terminators

Figure S2. The effect of terminator insertion upstream of chromosomally-integrated DNA on transcriptional read-through from chromosomal promoters. (A) Detail showing the insertion of terminators into integration plasmid pCK306 upstream of the *rhaBAD* promoter. The resulting constructs pCK351, pCK353, pCK354 and pCK355 were integrated into the *Synechocystis* genome adjacent to the *ndhB* gene. (B) To test for transcriptional insulation from chromosomal promoters after integration, *Synechocystis* cells containing either pCK351, 353, 354 or 355 (each with one of four terminators inserted upstream of *rhaBAD* promoter) were cultured in BG11 media supplemented with kanamycin and no L-rhamnose, in photoautotrophic conditions and constant light. The fluorescence intensity of 10,000 cells measured after 192 h using flow cytometry and compared to wild-type and *Synechocystis* cells lacking YFP entirely and cells containing pCK306 (no terminator, *rhaBAD* promoter, YFP). (C) Equivalent experiment to (B) but strains cultured in BG11 supplemented with 5 mM D-glucose (mixotrophic growth). (D) The same strains of *Synechocystis* were cultured in BG11 media supplemented kanamycin and L-rhamnose to a final concentration of 0.6 mg/ml in photoautotrophic conditions and constant light. The fluorescence intensity of 10,000 cells (arbitrary units) measured after 192 h using flow cytometry and compared to wild-type and *Synechocystis* cells (lacking YFP entirely) and cells containing pCK306 (no terminator, *rhaBAD* promoter, YFP). (E) Equivalent experiment to (D) but strains cultured in BG11 supplemented with 5 mM D-glucose (mixotrophic growth). Error bars shown are the standard deviation of the mean for three independent biological replicates.

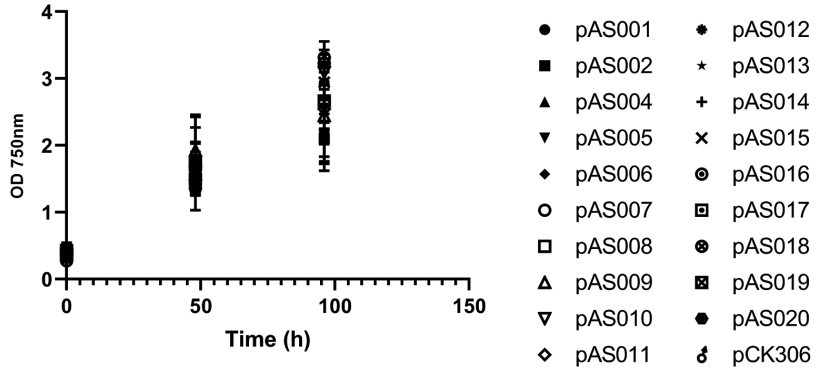

Figure S3. Growth of *Synechocystis* cells transformed with terminator plasmids, pAS001-pAS020. *Synechocystis* cells containing integrated terminator constructs from one of pAS001-002, pAS004-020 (terminator between *rhaBAD* promoter and RBS of YFP-encoding gene) or pCK306 (control, no terminator) were cultured in BG11 media supplemented with kanamycin and 0.6 mg/ml L-rhamnose in photoautotrophic conditions and constant light; and the optical density at 750 nm monitored over time. Error bars represent the standard deviation of the mean for three independent biological replicates.

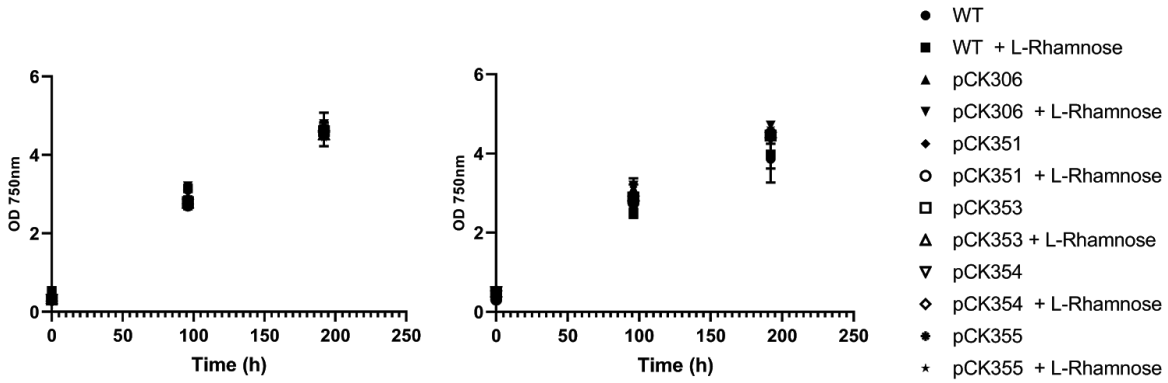

Figure S4. Growth of *Synechocystis* cells transformed with insulated plasmids pCK351, pCK353, pCK354 or pCK355. Wild-type (WT) *Synechocystis* cells, or cells containing integrated terminator constructs from one of pCK351, pCK353-355 plasmids (each with one of four Rho-independent terminators inserted upstream of the *rhaBAD* promoter) or pCK306 (control, no terminator) were cultured in BG11 media supplemented with kanamycin and 0 or 0.6 mg/ml L-rhamnose in photoautotrophic conditions and constant light; and the optical density at 750 nm monitored over time. Error bars represent the standard deviation of the mean for three independent biological replicates.

Terminator	Screening plasmid	Length (bp)	Sequence (5'-3')	ΔG (kcal/mol)	Origin	References
ECK120029600	pAS001	90	TTCAGCCAAAAAACTTAAGACCGCCGGTCTTGTCCACTACCTTGCAGTAATGCGGTGGACAGGATCGGCGGTTTTCTTTTCTCTTCTCAA	-42.00	*E. coli* K12	Chen *et al*., 2013
ECK120033737; *thrL* attenuator	pAS002	57	GGAAACACAGAAAAAAGCCCGCACCTGACAGTGCGGGCTTTTTTTTTCGACCAAAGG	-25.00	*E. coli* K12	Chen *et al*., 2013; Jeng *et al*., 1990
ECK120034435	pAS004	57	CTCGGTACCAAATTCCAGAAAAGAGACGCTGAAAAGCGTCTTTTTTCGTTTTGGTCC	-27.90	*E. coli* K12	Chen *et al*., 2013
L3S2P21	pAS005	61	CTCGGTACCAAATTCCAGAAAAGAGGCCTCCCGAAAGGGGGGCCTTTTTTCGTTTTGGTCC	-37.90	Synthetic	Chen *et al*., 2013
L3S2P56	pAS006	57	CTCGGTACCAAATTTTCGAAAAAAGACGCTGAAAAGCGTCTTTTTTCGTTTTGGTCC	-28.80	Synthetic	Chen *et al*., 2013
L3S2P51	pAS007	57	CTCGGTACCAAAAAAAAAAAAAAAGACGCTGAAAAGCGTCTTTTTTCGTTTTGGTCC	-24.90	Synthetic	Chen *et al*., 2013
L3S1P56	pAS008	52	TTTTCGAAAAAAGGCCTCCCAAATCGGGGGGCCTTTTTTATTGATAACAAAA	-23.40	Synthetic	Chen *et al*., 2013

Bba_B0015; *rrnB* terminator	pAS009	130	CCAGGCATCAAATAAAACGAAAGGCTCAG TCGAAAGACTGGGCCTTTCGTTTTATCTG TTGTTTGTCGGTGAACGCTCTCTACTAGA GTCACACTGGCTCACCTTCGGGTGGGCC TTTCTGCGTTTATA	-72.10	*E. coli* K12	Huang *et al*., 2010
ECK120035133	pAS010	43	ACTGATTTTTAAGGCGACTGATGAGTCGC CTTTTTTTTGTCT	-15.40	*E. coli* K12	Chen *et al*., 2013
ECK120017009	pAS011	44	GATCTAACTAAAAAGGCCGCTCTGCGGC CTTTTTTCTTTTCACT	-16.20	*E. coli* K12	Chen *et al*., 2013
ECK120015170	pAS012	47	ACAATTTTCGAAAAAACCCGCTTCGGCGG GTTTTTTTATAGCTAAAA	-20.10	*E. coli* K12	Chen *et al*., 2013
ECK120033736	pAS013	53	AACGCATGAGAAAGCCCCCGGAAGATCA CCTTCCGGGGGCTTTTTTATTGCGC	-37.80	*E. coli* K12	Chen *et al*., 2013
ECK120010799	pAS014	60	GTTATGAGTCAGGAAAAAAGGCGACAGA GTAATCTGTCGCCTTTTTTCTTTGCTTGCT TT	-33.60	*E. coli* K12	Chen *et al*., 2013
BBa_B0010; *rrnB* terminator	pAS015	80	CCAGGCATCAAATAAAACGAAAGGCTCAG TCGAAAGACTGGGCCTTTCGTTTTATCTG TTGTTTGTCGGTGAACGCTCTC	-42.90	*E. coli* K12	Geerts *et al*., 1995
Ω*groEL*	pAS016	89	GGTTTAGTAGACCGACTACCACTTTTCTC ATAAAATCCCAGGGAGGTTTCGGCCTCCC TTTTTTTCACTTGCTAAGCTCTCTTTCGTT T	-20.80	*Synechocystis* sp. PCC 6803	Jacobsen and Frigaard, 2014

T21	pAS017	74	ATTGAGCAAGTAGCAACACTATTCGCATA AGCTGCCGTTAGTGACTCTTAAGTTGCAA CGGTGGCTTTTTTTAT	-25.40	Bacteriophage T21	Cambray *et al*., 2013
M13 Central	pAS018	85	AAAGCAAGCTGATAAACCGATACAATTAA AGGCTCCTTTTGGAGCCTTTTTTTTTGGA GATTTTCAACATGAAAAAATTATTATT	-18.60	Bacteriophage M13	Cambray *et al*., 2013
ilvBN terminator	pAS019	36	AAGACCCCCGCACCGAAAGGTCCGGGGG TTTTTTTT	-24.40	*E. coli* K12	Chen *et al*., 2013; Cambray *et al*., 2013
ECK120010793	pAS020	34	TACGTAAAAACCCGCTTCGGCGGGTTTTT ACTTT	-24.40	*E. coli* K12	Chen *et al*., 2013; Cambray *et al*., 2013

Table S1. Terminators used in this study

Supplementary Materials and Methods

Plasmid Construction

A table of all plasmids and oligonucleotides (Table S2) is provided. Terminators were introduced as follows. Each terminator sequence was split in two at the hairpin-loop sequence and each part was included at the 5' end of oligonucleotides that were then used to amplify pCK306. PCR fragments were then ligated by blunt-end ligation using the New England Biolabs site-directed mutagenesis kit and sequence verified.

Name	Details
pCK306	Medium copy plasmid (p15A), with 2054 nucleotides of homology to the *Synechocystis* sp. PCC 6803 chromosome, allowing integration of DNA after the first 34 nucleotides of ssl0410 (adjacent to *ndhB*), antibiotic resistance gene *kanR* encoding an aminoglycoside phosphotransferase, the *rhaBAD* promoter from *E. coli*, a synthetic RBS and eYFP, the *E. coli rhaS* RBS and gene inserted downstream of the *kanR* gene. [1]
pAS001-20	Detailed in Table S1
pCK351	As pCK306 but with terminator ECK120034435 inserted upstream of *rhaBAD* promoter
pCK353	As pCK306 but with terminator ECK120015170 inserted upstream of *rhaBAD* promoter
pCK354	As pCK306 but with terminator ECK120010799 inserted upstream of *rhaBAD* promoter
pCK355	As pCK306 but with the *ilvBN* terminator inserted upstream of *rhaBAD* promoter
oligoAS001	ACTGCAAGGTAGTGGACAAGACCGGCGGTCTTAAGTTTTTTGGCTGAATA CGACCAGTCTAAAAAG Used in construction of pAS001
oligoAS002	AATGCGGTGGACAGGATCGGCGGTTTTCTTTTCTCTTCTCAAATGAATCG GGTAAGTTTATAATATAC Used in construction of pAS001
oligoAS003	AGGTGCGGGCTTTTTTCTGTGTTTCCTACGACCAGTCTAAAAAG Used in construction of pAS002
oligoAS004	GACAGTGCGGGCTTTTTTTTTCGACCAAAGGATGAATCGGGTAAGTTTAT AATATAC Used in construction of pAS002
oligoAS007	TCTGGAATTTGGTACCGAGTACGACCAGTCTAAAAAG

Used in construction of pAS004

oligoAS008 AAAGAGACGCTGAAAAGCGTCTTTTTTCGTTTTGGTCCATGAATCGGGTA
AGTTTATAATATAC
Used in construction of pAS004

oligoAS009 TCGGGAGGCCTCTTTTCTGGAATTTGGTACCGAGTACGACCAGTCTAAAA
AG
Used in construction of pAS005

oligoAS010 AAGGGGGGCCTTTTTTCGTTTTGGTCCATGAATCGGGTAAGTTTATAATAT
AC
Used in construction of pAS005

oligoAS011 TCAGCGTCTTTTTTCGAAAATTTGGTACCGAGTACGACCAGTCTAAAAAG
Used in construction of pAS006

oligoAS012 AAAGCGTCTTTTTTCGTTTTGGTCCATGAATCGGGTAAGTTTATAATATAC
Used in construction of pAS006

oligoAS013 TCAGCGTCTTTTTTTTTTTTTTTGGTACCGAGTACGACCAGTCTAAAAAG
Used in construction of pAS007

oligoAS014 AAAGCGTCTTTTTTCGTTTTGGTCCATGAATCGGGTAAGTTTATAATATAC
Used in construction of pAS007

oligoAS015 TTTGGGAGGCCTTTTTTCGAAAATACGACCAGTCTAAAAAG
Used in construction of pAS008

oligoAS016 TCGGGGGGCCTTTTTTATTGATAACAAAAATGAATCGGGTAAGTTTATAAT
ATAC
Used in construction of pAS008

oligoAS017 TCGACTGAGCCTTTCGTTTTATTTGATGCCTGGTACGACCAGTCTAAAAAG
Used in construction of pAS009

oligoAS018 AAGACTGGGCCTTTCGTTTTATCTGTTGTTTGTCGGTGAACGCTCTCTACT
AGAGTCACACTGGCTCACCTTCGGGTGGGCCTTTCTGCGTTTATAATGAA
TCGGGTAAGTTTATAATATAC
Used in construction of pAS009

oligoAS019 TCAGTCGCCTTAAAAATCAGTTACGACCAGTCTAAAAAG
Used in construction of pAS010

oligoAS020 TGAGTCGCCTTTTTTTTGTCTATGAATCGGGTAAGTTTATAATATAC
Used in construction of pAS010

oligoAS021 AGCGGCCTTTTTAGTTAGATCTACGACCAGTCTAAAAAG
Used in construction of pAS011

oligoAS022 CTGCGGCCTTTTTTCTTTTCACTATGAATCGGGTAAGTTTATAATATAC
Used in construction of pAS011

oligoAS023 AAGCGGGTTTTTTCGAAAATTGTTACGACCAGTCTAAAAAG
Used in construction of pAS012

oligoAS024 CGGCGGGTTTTTTTATAGCTAAAAATGAATCGGGTAAGTTTATAATATAC
Used in construction of pAS012

oligoAS025 ATCTTCCGGGGGCTTTCTCATGCGTTTACGACCAGTCTAAAAAG
Used in construction of pAS013

oligoAS026 CACCTTCCGGGGGCTTTTTTATTGCGCATGAATCGGGTAAGTTTATAATATAC
Used in construction of pAS013

oligoAS027 ACTCTGTCGCCTTTTTTCCTGACTCATAACTACGACCAGTCTAAAAAG
Used in construction of pAS014

oligoAS028 AATCTGTCGCCTTTTTTCTTTGCTTGCTTTATGAATCGGGTAAGTTTATAATATAC
Used in construction of pAS014

oligoAS029 TTCGACTGAGCCTTTCGTTTTATTTGATGCCTGGTACGACCAGTCTAAAAAG
Used in construction of pAS015

oligoAS030 AGACTGGGCCTTTCGTTTTATCTGTTGTTTGTCGGTGAACGCTCTCATGAATCGGGTAAGTTTATAATATAC
Used in construction of pAS015

oligoAS031 AAACCTCCCTGGGATTTTATGAGAAAAGTGGTAGTCGGTCTACTAAACCTACGACCAGTCTAAAAAG
Used in construction of pAS016

oligoAS032 CGGCCTCCCTTTTTTTCACTTGCTAAGCTCTCTTTCGTTTATGAATCGGGTAAGTTTATAATATAC
Used in construction of pAS016

oligoAS033 AGAGTCACTAACGGCAGCTTATGCGAATAGTGTTGCTACTTGCTCAATTACGACCAGTCTAAAAAG
Used in construction of pAS017

oligoAS034 TAAGTTGCAACGGTGGCTTTTTTTATATGAATCGGGTAAGTTTATAATATAC
Used in construction of pAS017

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Used in construction of pAS018

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Used in construction of pAS018

oligoAS037 TCGGTGCGGGGGTCTTTACGACCAGTCTAAAAAG

	Used in construction of pAS019
oligoAS038	AAGGTCCGGGGGTTTTTTTTATGAATCGGGTAAGTTTATAATATAC Used in construction of pAS019
oligoAS039	AAGCGGGTTTTTACGTATACGACCAGTCTAAAAAG Used in construction of pAS020
oligoAS040	CGGCGGGTTTTTACTTTATGAATCGGGTAAGTTTATAATATAC Used in construction of pAS020

Table S2. Plasmids and oligonucleotides used in this study

Supplementary References

1. Kelly, C.L.; Taylor, G.M.; Hitchcock, A.; Torres-Méndez, A.; Heap, J.T. A Rhamnose-Inducible System for Precise and Temporal Control of Gene Expression in Cyanobacteria. *ACS Synth. Biol.* **2018**, *7*, 1056–1066.