

Supplementary Materials

# Optimization of Milbemycin Component Ratio by Coordinating Acyl-Coenzyme A Supply Pathways in *Streptomyces bingchenggensis*

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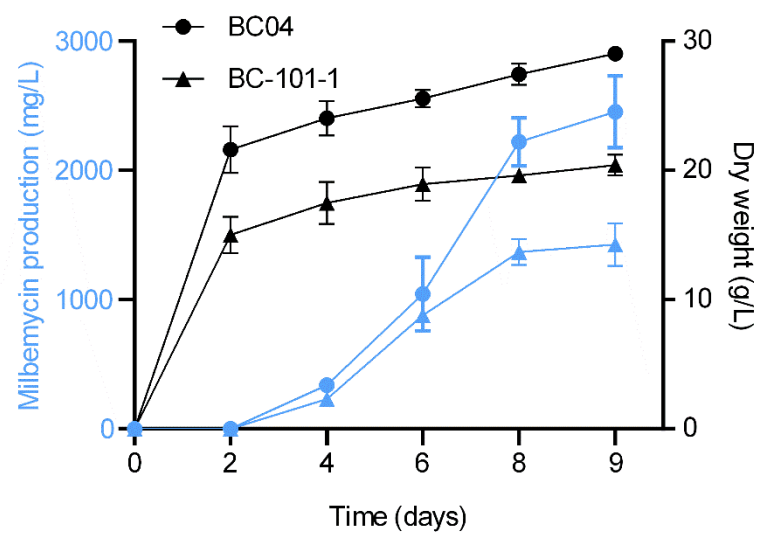
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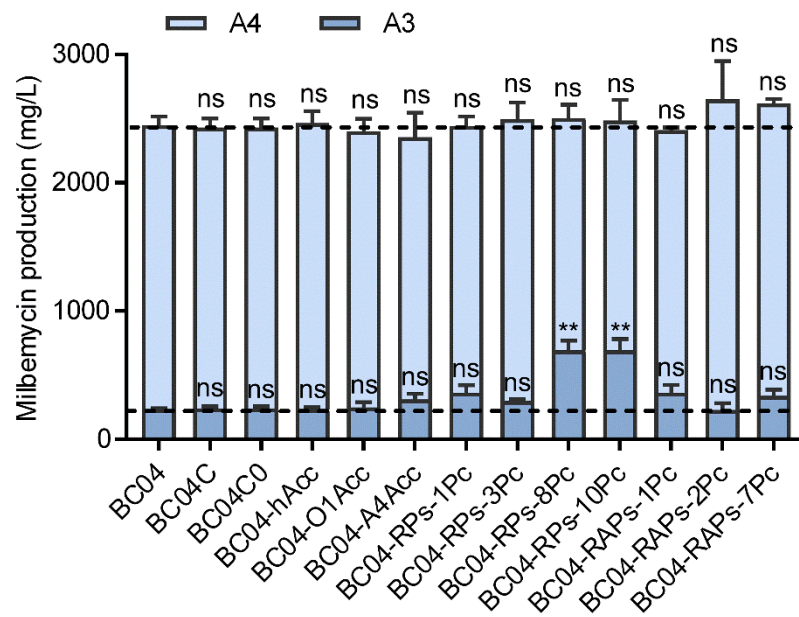
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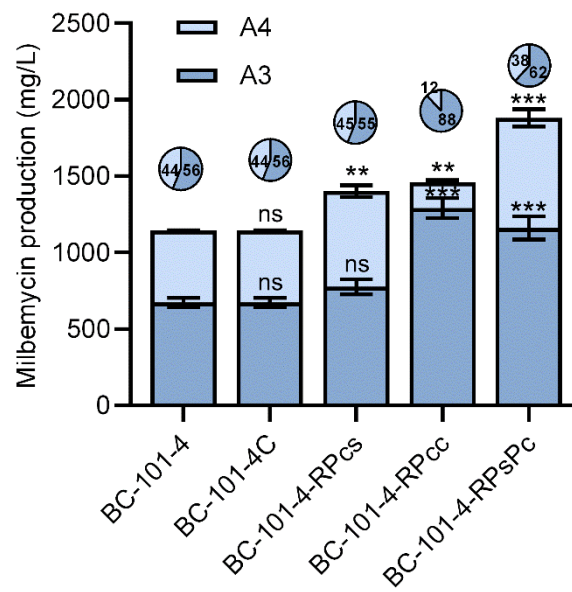
† These authors contributed equally to this work.



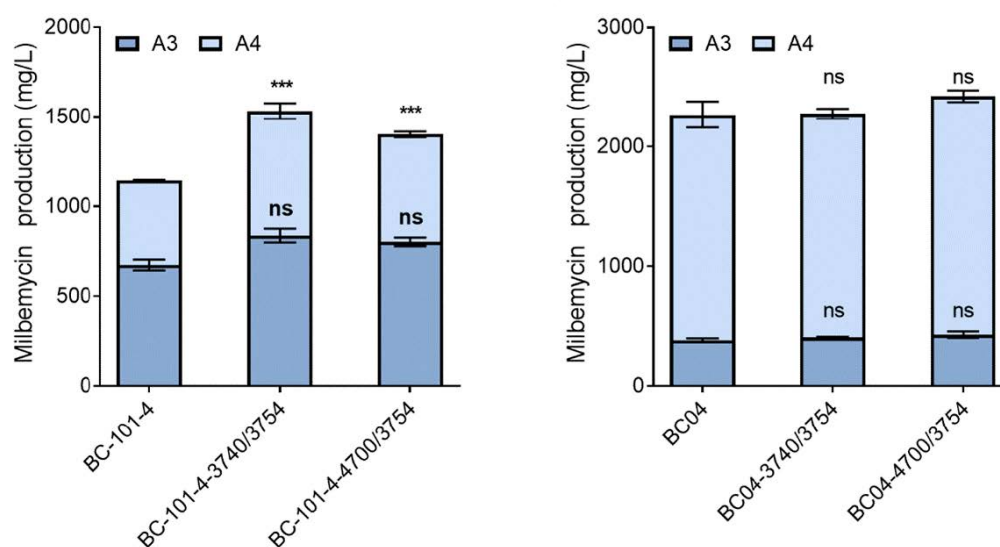
**Figure S1. Time-course curves of cell growth and milbemycin production.** Data shown are the average and s.d. of three independent experiments.



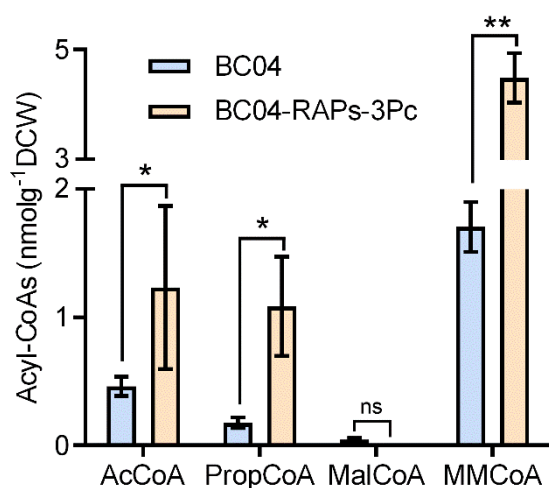
**Figure S2. Mutant strains without significant effects on milbemycin production in the precursor coordination strategy.** Data shown are the average and s.d. of three independent experiments. Differences are analyzed by Student's *t*-test,  $p < 0.05$  is considered statistically significant. Levels of significance are \*\*  $p < 0.01$ , "ns" means no significant difference.



**Figure S3. The milbemycin production of PCS and PCC overexpression strains in BC-101-4.** Data shown are the average and s.d. of three independent experiments. Differences are analyzed by Student's *t*-test,  $p < 0.05$  is considered statistically significant. Levels of significance are \*\*\*  $p < 0.001$ , \*\*  $p < 0.01$ , “ns” means no significant difference.



**Figure S4. The milbemycin production of MCM and MCE overexpression strains in BC-101-4 and BC04.** Data shown are the average and s.d. of three independent experiments. Differences are analyzed by Student's *t*-test,  $p < 0.05$  is considered statistically significant. Levels of significance are \*\*\*  $p < 0.001$ , "ns" means no significant difference.



**Figure S5. The precursor concentration of strain BC04 and BC04-RAPs-3Pc.** Data shown are the average and s.d. of three independent experiments. Differences are analyzed by Student's *t*-test,  $p < 0.05$  is considered statistically significant. Levels of significance are \*\*  $p < 0.01$ , \*  $p < 0.05$ , "ns" means no significant difference.

**Table S1. Strains and Plasmids used in this work**

Name	Description	Source
<b>Strains</b>		
<i>Escherichia coli</i>		
<i>E. coli</i> JM109	General cloning host for plasmid manipulation	Novagen
ET12567 (pUZ8002)	Donor strain for conjugation between <i>E. coli</i> and <i>Streptomyces</i>	[1]
<i>Streptomyces bingchenggensis</i>		
BC-101-4	Low-yielding strain	[2]
BC04	High-yielding strain of milbemycin A3/A4	[3]
BC-101-4C	BC-101-4 integrated with a copy of pSET152	This work
BC04C0	BC04 integrated with a copy of pIJ10500	This work
BC04C	BC04 integrated with a copy of pSET152	This work
BC04-hAcc	BC04 integrated a copy of genes <i>sbi_06761</i> , <i>sbi_03526</i> and <i>sbi_03527</i> driven by the <i>hrdB</i> promoter	This work
BC04-O1Acc	BC04 integrated a copy of genes <i>sbi_06761</i> , <i>sbi_03526</i> and <i>sbi_03527</i> driven by the promoter region of gene <i>orf1</i>	This work
BC04-A1Acc	BC04 integrated a copy of genes <i>sbi_06761</i> , <i>sbi_03526</i> and <i>sbi_03527</i> driven by the promoter region of gene <i>milA1</i>	This work
BC04-A2Acc	BC04 integrated a copy of genes <i>sbi_06761</i> , <i>sbi_03526</i> and <i>sbi_03527</i> driven by the promoter region of gene <i>milA2</i>	This work
BC04-A4Acc	BC04 integrated a copy of genes <i>sbi_06761</i> , <i>sbi_03526</i> and <i>sbi_03527</i> driven by the promoter region of gene <i>milA4</i>	This work
BC04-RAcc	BC04 integrated a copy of genes <i>sbi_06761</i> , <i>sbi_03526</i> and <i>sbi_03527</i> driven by the promoter region of gene <i>milR</i>	This work

BC04-FAcc	BC04 integrated a copy of genes <i>sbi_06761</i> , <i>sbi_03526</i> and <i>sbi_03527</i> driven by the promoter region of gene <i>milF</i>	This work
BC-101-4-3740/3754	BC-101-4 integrated a copy of <i>sbi_03740</i> and <i>sbi_03754</i> driven by the promoter region of gene <i>milR</i>	This work
BC-101-4-4700/3754	BC-101-4 integrated a copy of <i>sbi_04700</i> and <i>sbi_03754</i> driven by the promoter region of gene <i>milR</i>	This work
BC04-3740/3754	BC04 integrated a copy of <i>sbi_03740</i> and <i>sbi_03754</i> driven by the promoter region of gene <i>milR</i>	This work
BC04-4700/3754	BC04 integrated a copy of <i>sbi_04700</i> and <i>sbi_03754</i> driven by the promoter region of gene <i>milR</i>	This work
BC-101-4-RPcs	BC-101-4 integrated a copy of gene <i>sbi_01198</i> driven by the <i>milR</i> promoter	This work
BC-101-4-RPcc	BC-101-4 integrated a copy of genes <i>sbi_04611</i> and <i>sbi_04601</i> driven by the <i>milR</i> promoter	This work
BC-101-4-RPsPc	BC-101-4 integrated a copy of genes <i>sbi_01198</i> , <i>sbi_04611</i> and <i>sbi_04601</i> driven by the <i>milR</i> promoter	This work
BC04-RPcs	BC04 integrated a copy of gene <i>sbi_01198</i> driven by the <i>milR</i> promoter	This work
BC04-RPcc	BC04 integrated a copy of genes <i>sbi_04611</i> and <i>sbi_04601</i> driven by the <i>milR</i> promoter	This work
BC04-RPsPc	BC04 integrated a copy of genes <i>sbi_01198</i> , <i>sbi_04611</i> and <i>sbi_04601</i> driven by the <i>milR</i> promoter	This work



BC04-RPs- <i>n</i> Pc	BC04 integrated a copy of gene <i>sbi_01198</i> driven by the <i>milR</i> promoter, <i>sbi_04611</i> and <i>sbi_04601</i> driven by native temporal promoter regions of genes, <i>n</i> (1-10) indicates the promoter of genes <i>sbi_00972</i> , <i>sbi_05175</i> , <i>sbi_05992</i> , <i>sbi_04950</i> , <i>sbi_09717</i> , <i>sbi_04323</i> , <i>sbi_04187</i> , <i>sbi_09292</i> , <i>sbi_03281</i> , <i>sbi_03269</i> , respectively	This work
BC04-RAPs- <i>n</i> Pc	BC04 integrated a copy of genes <i>sbi_06761</i> , <i>sbi_03526</i> , <i>sbi_03527</i> and <i>sbi_01198</i> driven by the <i>milR</i> promoter, <i>sbi_04611</i> and <i>sbi_04601</i> driven by the native temporal promoter regions of genes, <i>n</i> (1-10) indicates the promoter of genes <i>sbi_00972</i> , <i>sbi_05175</i> , <i>sbi_05992</i> , <i>sbi_04950</i> , <i>sbi_09717</i> , <i>sbi_04323</i> , <i>sbi_04187</i> , <i>sbi_09292</i> , <i>sbi_03281</i> , <i>sbi_03269</i> , respectively	This work
<b>Plasmids</b>		
pSET152	Am <sup>r</sup> , integrative <i>E. coli-Streptomyces</i> shuttle vector	[1]
pIJ10500	Hyp <sup>r</sup> , containing ΦBT1 integrase gene and integration site attB	[1]
pIJ10500::Porf1::ACC	Hyp <sup>r</sup> , derived from pIJ10500, containing the genes <i>sbi_06761</i> , <i>sbi_03526</i> and <i>sbi_03527</i> driven by the promoter region of gene <i>orf1</i>	This work
pIJ10500::PmilA1::ACC	Hyp <sup>r</sup> , derived from pIJ10500, containing the genes <i>sbi_06761</i> , <i>sbi_03526</i> and <i>sbi_03527</i> driven by the promoter region of gene <i>milA1</i>	This work
pIJ10500::PmilA2::ACC	Hyp <sup>r</sup> , derived from pIJ10500, containing the genes <i>sbi_06761</i> , <i>sbi_03526</i> and <i>sbi_03527</i> driven by the promoter region of gene <i>milA2</i>	This work
pIJ10500::PmilA4::ACC	Hyp <sup>r</sup> , derived from pIJ10500, containing the genes <i>sbi_06761</i> , <i>sbi_03526</i> and <i>sbi_03527</i> driven by the promoter region of gene <i>milA4</i>	This work
pIJ10500::PmilR::ACC	Hyp <sup>r</sup> , derived from pIJ10500, containing the genes <i>sbi_06761</i> , <i>sbi_03526</i> and <i>sbi_03527</i> driven by the promoter region of gene <i>milR</i>	This work
pIJ10500::PmilF::ACC	Hyp <sup>r</sup> , derived from pIJ10500, containing the genes <i>sbi_06761</i> , <i>sbi_03526</i> and <i>sbi_03527</i> driven by the promoter region of gene <i>milF</i>	This work

pSET152::PmilR::PCS	Am <sup>r</sup> , derived from pSET152, containing the gene <i>sbi_01198</i> driven by the <i>milR</i> promoter	This work
pSET152::PmilR::PCC	Am <sup>r</sup> , derived from pSET152, containing the genes <i>sbi_04611</i> and <i>sbi_04601</i> driven by the <i>milR</i> promoter	This work
pSET152::PmilR::PCS::PCC	Am <sup>r</sup> , derived from pSET152, containing the gene <i>sbi_01198</i> , <i>sbi_04611</i> and <i>sbi_04601</i> driven by the <i>milR</i> promoter	This work
pSET152::PmilR::sbi_03740::sbi_03754	Am <sup>r</sup> , derived from pSET152, containing the genes <i>sbi_03740</i> and <i>sbi_3754</i> driven by the <i>milR</i> promoter	This work
pSET152::PmilR::sbi_04700::sbi_03754	Am <sup>r</sup> , derived from pSET152, containing the genes <i>sbi_04700</i> and <i>sbi_3754</i> driven by the <i>milR</i> promoter	This work
pSET152::PmilR::PCS::PnPCC	Am <sup>r</sup> , derived from pSET152, a series of plasmids containing the gene <i>sbi_01198</i> driven by the <i>milR</i> promoter and gene <i>PCC</i> driven by the native temporal promoter regions of genes, <i>n</i> (1-10) indicates the genes <i>sbi_00972</i> ( <i>P1</i> ), <i>sbi_05175</i> ( <i>P2</i> ), <i>sbi_05992</i> ( <i>P3</i> ), <i>sbi_04950</i> ( <i>P4</i> ), <i>sbi_09717</i> ( <i>P5</i> ), <i>sbi_04323</i> ( <i>P6</i> ), <i>sbi_04187</i> ( <i>P7</i> ), <i>sbi_09292</i> ( <i>P8</i> ), <i>sbi_03281</i> ( <i>P9</i> ), <i>sbi_03269</i> ( <i>P10</i> ), respectively	This work
pSET152::PmilR::ACC::PCS::PnPCC	Am <sup>r</sup> , derived from pSET152, a series of plasmids containing the gene <i>sbi_06761</i> , <i>sbi_03526</i> , <i>sbi_03527</i> and <i>sbi_01198</i> driven by the <i>milR</i> promoter, gene <i>PCC</i> driven by the native temporal promoter regions of genes, <i>n</i> (1-10) indicates the genes <i>sbi_00972</i> ( <i>P1</i> ), <i>sbi_05175</i> ( <i>P2</i> ), <i>sbi_05992</i> ( <i>P3</i> ), <i>sbi_04950</i> ( <i>P4</i> ), <i>sbi_09717</i> ( <i>P5</i> ), <i>sbi_04323</i> ( <i>P6</i> ), <i>sbi_04187</i> ( <i>P7</i> ), <i>sbi_09292</i> ( <i>P8</i> ), <i>sbi_03281</i> ( <i>P9</i> ), <i>sbi_03269</i> ( <i>P10</i> ), respectively	This work

**Table S2. Primers used in this work**

Primers	Sequence (5'-3') <sup>a</sup>	Usage
accA2-F	ATGCAAAAGGTGCTCATCGCCAATC	For amplification of <i>sbi_06761</i>
accA2-R	TCAGTCTTTGATTTTCGCAGATGACG	
accBE-F	GCCGTCATCTGCGAAATCAAAGACTGAGGGAACGTACCGTCATTGCATGA	For amplification of <i>sbi_03526-sbi_03527</i>
accBE-R	TCGTTAGTTAGGCTA <u>ACTAGTTC</u> AGCCCTGCCAGCTGTGC	
Porf1-F	GTCCTCGAGAGGCCT <u>CATATG</u> GTCAGCACCCCTGCTACATCATCAA	For amplification of promoter <i>P<sub>orf1</sub></i>
Porf1-R	GATTGGCGATGAGCACCTTTTGCATGGCCCTCCAGGACTGCTGTCA	
PmilA1-F	GTCCTCGAGAGGCCT <u>CATATG</u> GTCAGCACCCCTGCTACATCATCAA	For amplification of promoter <i>P<sub>milA1</sub></i>
PmilA1-R	GATTGGCGATGAGCACCTTTTGCATGGCCCTCCAGGACTGCTGTCA	
PmilA2-F	GTCCTCGAGAGGCCT <u>CATATG</u> GTCAGCACCCCTGCTACATCATCAA	For amplification of promoter <i>P<sub>milA2</sub></i>
PmilA2-R	GATTGGCGATGAGCACCTTTTGCATGGCCCTCCAGGACTGCTGTCA	
PmilA4-F	GTCCTCGAGAGGCCT <u>CATATG</u> GTCAGCACCCCTGCTACATCATCAA	For amplification of promoter <i>P<sub>milA4</sub></i>
PmilA4-R	GATTGGCGATGAGCACCTTTTGCATGGCCCTCCAGGACTGCTGTCA	
PmilR-F	GTCCTCGAGAGGCCT <u>CATATG</u> GTCAGCACCCCTGCTACATCATCAA	For amplification of promoter <i>P<sub>milR</sub></i>
PmilR-R	GATTGGCGATGAGCACCTTTTGCATGGCCCTCCAGGACTGCTGTCA	
PmilF-F	GTCCTCGAGAGGCCT <u>CATATG</u> GTCAGCACCCCTGCTACATCATCAA	For amplification of promoter <i>P<sub>milF</sub></i>
PmilF-R	GATTGGCGATGAGCACCTTTTGCATGGCCCTCCAGGACTGCTGTCA	
SBI_01198-F	ATGCCGGTGACCAGGCCGCT	For amplification of <i>sbi_01198</i>
SBI_01198-R	AACAGCTATGACATGATTAC <u>GAATTCT</u> CACGGGGTGTGACCGGCG	
SBI_01198E-R	TTTCGGA <u>CTAGTTC</u> ACGGGGTGTGACCGGCG	
SBI_04611-F	CACACCCCGTGAAGTACTGTCGAAA <u>AGTACTT</u> CAAGGAGGGAG CCATCGTGC	For amplification of <i>sbi_04611</i>
SBI_04611-R	TCAGTCCTTGATTTTCGCAGATGACG	
SBI_04601-F	CGTCATCTGCGAAATCAAGGACTGAGAGGACGAGCAGGAGCCGCATC	

SBI_04601-R	AACAGCTATGACATGATTACGAATTCCTACAGCGGGATGTTGCCGTGCTTC	For amplification of <i>sbi_04601</i>
PhrdB-F	GGGCTGCAGGTCGACTCTAGACCGCCTTCCGCCGGAACG	For amplification of <i>hrdB</i>
PhrdB-R	AGCGGCCTGGTCACCGGCATGAACAACCTCTCGGAACGTTGAAAA	promoter
PSBI_00972-F	CGCCGGTCACACCCCGTGAAGTACTCGCTCTGCCTGCCGCGCTGGTCTGC	For amplification of
PSBI_00972-R	ACGATGGCTCCCTCCTTGAAGTACTGACACGCCCTCCTGCCACGCCATCC	promoter P <sub>sbi_00972</sub>
PSBI_05175-F	CGCCGGTCACACCCCGTGAAGTACTAGGTGCCGCCCTGCGGGT	For amplification of
PSBI_05175-R	ACGATGGCTCCCTCCTTGAAGTACTGGACTTCTCTCCCCGATTTCCTC	promoter P <sub>sbi_05175</sub>
PSBI_05992-F	CGCCGGTCACACCCCGTGAAGTACTGCCAGGTGAGCATGCTCGC	For amplification of
PSBI_05992-R	ACGATGGCTCCCTCCTTGAAGTACTTGGTCTGTCCCGTTCTTCTTGAGAA	promoter P <sub>sbi_05992</sub>
PSBI_04950-F	CGCCGGTCACACCCCGTGAAGTACTTACGCGACTTCGAGCGGTCC	For amplification of
PSBI_04950-R	ACGATGGCTCCCTCCTTGAAGTACTGCCTCCCTGTTGTGCTTCGC	promoter P <sub>sbi_04950</sub>
PSBI_09717-F	CGCCGGTCACACCCCGTGAAGTACTACGAGGTTCCGGAGCCCAAGTCGG	For amplification of
PSBI_09717-R	ACGATGGCTCCCTCCTTGAAGTACTCGCCGTATTCCCCTGCCCTCGG	promoter P <sub>sbi_09717</sub>
PSBI_04323-F	CGCCGGTCACACCCCGTGAAGTACTGCCAGGTGAGCATGCTCGC	For amplification of
PSBI_04323-R	ACGATGGCTCCCTCCTTGAAGTACTGGAAGTCTCCCGTCACGAGGAGG	promoter P <sub>sbi_04323</sub>
PSBI_04187-F	CGCCGGTCACACCCCGTGAAGTACTGTACTACTTCTGCGAGGTCTTCTGG	For amplification of
PSBI_04187-R	ACGATGGCTCCCTCCTTGAAGTACTGGGTGGACGAACCCCTC	promoter P <sub>sbi_04187</sub>
PSBI_09292-F	CGCCGGTCACACCCCGTGAAGTACTCGACCGAGCCGCCGACTGAGC	For amplification of
PSBI_09292-R	ACGATGGCTCCCTCCTTGAAGTACTGCGCGCCGCTCTCCCATGTCTT	promoter P <sub>sbi_09292</sub>
PSBI_03281-F	CGCCGGTCACACCCCGTGAAGTACTGGTCGTAGCGAACGGTGGCGATGAA	For amplification of
PSBI_03281-R	ACGATGGCTCCCTCCTTGAAGTACTGGTGTTGCCCTTCAGGTGCGGGGT	promoter P <sub>sbi_03281</sub>
PSBI_03269-F	CGCCGGTCACACCCCGTGAAGTACTTGGACCAGCGGAAGGTCCCGGTCA	For amplification of
PSBI_03269-R	ACGATGGCTCCCTCCTTGAAGTACTGTGCGCCCCCTCGTCGTGGGTCCGT	promoter P <sub>sbi_03269</sub>
SBI_03740-F	CATGATCCGTGACAGCAGTCCTGGAGGGCCTTGTCCGGGGACCCCGTGCACCCGGTCT	

SBI_03740-R	CTAAAAGCCCGCCGGCTCCGTATAGCTGCC	For amplification of <i>sbi_03740</i>
SBI_04700-F	<b>CATGATCCGTGACAGCAGTCCTGGAGGGCC</b> ATGGCGCGCGATTTCGGAGTCGGGCTTCC	For amplification of <i>sbi_04700</i>
SBI_04700-R	TCAGAAGGCATCGGCGGGGACATACGTACC	
SBI_03754-F1	<b>GGCAGCTATACGGAGCCGGCGGGCTTTTAG</b> ATGCTGACCAGAATCGACCACATCGGGA	For amplification of <i>sbi_03754</i>
SBI_03754-F2	ATGCTGACCAGAATCGACCACATCGGGA	
SBI_03754-R	<b>GCCAGTGCCAAGCTTGGGCTGCAGGTCGAC</b> <u>GAATTCT</u> CAGCTCTTCGTACGGTCCTCGGA GGTG	

<sup>a</sup> Bold type characters indicate homologous fragments, and the underline characters indicate restriction enzyme sit.

**Table S3. Temporal promoter and their strengths in BC-101-4 and BC04 used in this work**

Promoter naming	Gene ID	18h	2d	3d	4d	6d	8d
BC-101-4							
P1	<i>sbi_00972</i>	4.090872	6.878499	7.913545	8.135700	7.392623	7.863440
P2	<i>sbi_05175</i>	4.567386	6.987736	7.256083	7.499217	6.404183	6.531797
P3	<i>sbi_05992</i>	5.773929	6.004744	6.764759	7.678828	7.747337	7.756427
P4	<i>sbi_04950</i>	4.828707	6.643884	7.274602	8.300183	7.800410	8.155011
P5	<i>sbi_09717</i>	6.101785	7.260918	7.648030	7.757221	7.487762	7.850243
P6	<i>sbi_04323</i>	7.153607	8.084553	9.166491	9.710273	9.433166	9.679818
P7	<i>sbi_04187</i>	8.045577	8.524642	9.538922	9.928432	9.983877	10.180663
P8	<i>sbi_09292</i>	8.237638	9.919695	10.545469	10.845125	10.764878	11.238493
P9	<i>sbi_03281</i>	9.116478	9.279103	10.046194	10.284767	10.390846	10.682231
P10	<i>sbi_03269</i>	9.693670	12.088699	13.020067	13.560289	13.412348	13.176568
BC04							
P1	<i>sbi_00972</i>	3.396891	6.155781	7.840112	8.179029	8.171165	8.12389
P2	<i>sbi_05175</i>	4.257788	6.847953	7.934483	8.424753	8.156127	8.370843
P3	<i>sbi_05992</i>	4.989388	6.953562	6.89001	7.631178	8.381467	8.609141
P4	<i>sbi_04950</i>	5.358833	7.874984	7.402656	8.342823	8.665964	9.106632
P5	<i>sbi_09717</i>	5.945079	7.234325	7.853852	7.809499	8.057441	8.009625
P6	<i>sbi_04323</i>	6.547695	8.220023	9.19416	9.177915	9.203987	9.266149
P7	<i>sbi_04187</i>	7.431193	9.543858	9.471892	9.955108	9.850662	10.03478
P8	<i>sbi_09292</i>	8.073007	10.61315	11.41202	11.33908	11.33302	11.53879
P9	<i>sbi_03281</i>	8.483576	9.060209	10.24006	10.3648	10.64543	10.53407
P10	<i>sbi_03269</i>	9.932167	12.87957	13.1092	13.6984	13.86001	13.99325

Data are given as log2-transformed of FPKM.

## References

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