Optimization of Constitutive Promoters Using a Promoter-Trapping Vector in *Burkholderia pyrrocinia* JK-SH007

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SUPPLEMENTARY MATERIAL

Supplementary Tables

Supplementary Figures

Table S1. Primers used in this study.

| Primers	Primer sequences (5'-3')	Source
P4-F	*GGCCTTTCGTTTTATAAGCTT*TTCCTCCTCTAGGGAGGTG	pBBR1-GM^r-P4-Luc-TP^r
P4-R	*TGGTAAATTACTCGAGGCGCC*AACTGCGGTAGCTGCAATGA	
P9-F	*GGCCTTTCGTTTTATAAGCTT*GGCTCACCGGCCGGGC	pBBR1-GM^r-P9-Luc-TP^r
P9-R	*TGGTAAATTACTCGAGGCGCCC*CACAGTACCGCGAAGGAT	
P10-F	GGCCTTTCGTTTTATAAGCTTCAACCGGCTGCGCATTCC	pBBR1-GM^r-P10-Luc-TP^r
P10-R	*TGGTAAATTACTCGAGGCGCC*GGTGTCCTCGGATTTGTGC	
P14-F	*GGCCTTTCGTTTTATAAGCTT*TTTTCCGCACGCCGGCGCCG	pBBR1-GM^r-P14-Luc-TP^r
P14-R	*TGGTAAATTACTCGAGGCGCCC*GGGCACGCTCCTGTTGG	
P15-F	*GGCCTTTCGTTTTATAAGCTT*GCGGACGGTTCCAAAAACG	pBBR1-GM^r-P15-Luc-TP^r
P15-R	*TGGTAAATTACTCGAGGCGCC*ACAACCACCTTCGAATCCTTT	
P17-F	*GGCCTTTCGTTTTATAAGCTT*CCCGCAGCAGCGAACCC	pBBR1-GM^r-P17-Luc-TP^r
P17-R	*TGGTAAATTACTCGAGGCGCC*GTTGTTCTCCTTCAGGTTCAG	
P18-F	*GGCCTTTCGTTTTATAAGCTT*CGACTTGATCGAACCCGTG	pBBR1-GM^r-P18-Luc-TP^r
P18-R	*TGGTAAATTACTCGAGGCGCC*GTCCGTTTCTTGCTGATTCTG	
P19-F	*GGCCTTTCGTTTTATAAGCTT*CCCGCCCGCAAGCGCC	pBBR1-GM^r-P19-Luc-TP^r
P19-R	*TGGTAAATTACTCGAGGCGCC*AAGATTCCGTTTTTGCATCGTT	
PT1-F	*TCGACCTCGAGGGGGGGCCC*GGTACCCAGCTTTTGTTCCCTTTA	pBBR1-GM^r-PRPL larger fragment [1]
PT1-R	CAGCCCGGGGGATCCACTAGT*TCTAGAGCGGCCGCCA*	
PRPL-F	GCGCGTAATACGACTCACTATAG	*PrPl* smaller fragment [2]
PRPL-R	*TTGGCATCTTCCATG*TATGAGTCTCCAGTTTGTTTCAGTT	
Luc-TP^r-F	*CTGAAACAAACTGGAGACTCATA*CATGGAAGATGCCAAAAACATTAAG	Luc-TP^r smaller fragment [2]
Luc-TP^r-R	*TAAAGGGAACAAAAGCTGGGTACC*GGGCCCCCCCTCGAG	
PTC1-F	GCGCGTAATACGACTCACTATAG	pBBR1-GM^r-PRPL-Luc-TP^r colony PCR check
PTC1-R	GGCTCGTATGTTGTGTGGAA	
PT2-F	GGCGCCTCGAGTAATTTA	pBBR1-GM^r -Luc-TP^r larger fragment
PT2-R	AAGCTTATAAAACGAAAGGCC	
PTC2-F	GCGCGTAATACGACTCACT	pBBR1-GM^r-Promoter-Luc-TP^r colony PCR check
PTC2-R	ATGTTGCATCACCTTCACC	
PT3-F	GAATTCTTTGCTTGTCGCC	pBBR1-GM^r -GFP-TP^r larger fragment *gfp* fragment
PT3-R	TATGAGTCTCCAGTTTGTTTCAGTT	
gfp-F	*AACAAACTGGAGACTCATA*ATGAGTAAAGGAGAAGAACT	
gfp-R	*TTATGGCGACAAGCAAAGAATTC*TTATTTGTATAGTTCATCCAT	
rfp-F	*TGAAACAAACTGGAGACTCATA*ATATAGCATGCGGGTCTTCCAA	*rfp* fragment
rfp-R	*ATTATGGCGACAAGCAAAGAATTC*TTAAAGGAACAGATGGTGGC	
qRT-*luc*-F	GCTCAGCAAGGAGGTAGGTG	qPCR for *luc* gene
qRT-*luc*-R	CCAGTGTCTTACCGGTGTCC	
qRT-*pyr*G-F	AGTCACCCTCCTCAAACTCG	qPCR for *pyrG* gene [3]
qRT-*pyr*G-R	TCGTGAAGTTGTTGGCCTTG	
qRT-16SrRNA-F	GATGACCAGCCACACTGGAA	qPCR for *E.coli* 16SrRNA gene
qRT-16SrRNA-R	GGAGTTAGCCGGTGCTTCTT	
qRT-16SrRNA-F	CCTACCAAGGCGACGATCAG	qPCR for *B.multivorans* 16SrRNA gene
qRT-16SrRNA-R	CAAAATTCCCCACTGCTGCC	

The homology arms are in italic.

Table S2. Promoter sequences selected in this study.

| Promoter	Promoter sequence
P4	<u>CAT</u>TTCCTCCTCTAGGGAGGTGGGCAGGGTCTCTTTGGCGAATTGATCCATGTATCCGTATCGTTTCGGAGCGAAGACGGCGAACGCCTTTCGCGTGGGCGCTGCATGCGCAACGCAACGCGAGATTCTAACATGCGCCCATCAGCCGCAAATTCGCCTCCCCCTCTATATATAAATAGCGAAAATCGCGGCGGCGGCGGACCGGCCGGAACCGCACGCGACTCGACGCCACCGTTGATCGCGCCCGGTTTACCGGCGATCCCACCCGGTTTGTTTCACGCGCACGAGTAAACAGTTGCCCGCACCCGCGCAATTTCGCCGGCGCACTCTTGACAATCTCTAAAAGTTGCGGCAGGCCGGTGTTGCGCATGCACCACAAAGTTGAGGGGATCTTAGGGTGGGGAGGATTTTTTTTCGTAGGAAGGGAACGATATGGCAAAATGCCAACGCACTGAAAGTTAGACGCTGCTCGAAGTCTACACAGAGCGGTGCCGCGTTTTTTGTGCCGCACCAATGTTATACTTCGAGCAATGTATGACTTGTCACCGTCAACAGGCTCGCAGTAAACCTGCGGTAATCTCAATTTCGAGAGGAGAAATATGAATAAACTTTCAAAGCTCGCGTTCATTGCAGCTACCGCAGTT<u>ATG</u>
P9	<u>CAC</u>GGCTCACCGGCCGGGCCGGGGCTGACGCGCGCCCCGGCAGTCCCGATCTGGCATACGGCCCGCGATCGCGTCGATCGCACCTCTGGCACGAGGCGGCGGCAGCCGCCGAGGACGGGCGTCCGCGGCACAGGATCGGGAAGTTCCCAGTGTGGGGCCCGATCGCGTGCAAGTCTGTCCCGAAAGTTTCGTGAAAGTGTGCCGATTTGTGCGCGATTGTCGGAGTGTGTCGTTCCGGCCGCGCCGTCAAAACGCTGAAACATCGGGTCGGACCGCATCCCGGCAGTGCCGGATTGCGTCGATCGGCCCGGTTCCGGCGGCGGCCCGAGCGCGACAGATCGACGCGCCCCCGGCCATGTCATCCCATGTCTCGAACGTCGCCGATGCTTGCGTCGACACCGCAAGCGCTGATGCCACGTGCGTTTGCGCGCGCCGCCGAAATGTCCGCGGATCGTCAGACGTCGTTTCATCTGAAACCGCAATGGACTCAGTATCGATTTATTGCGGATTATCGCTTGCGTTTCACACCAGTCTATCTAGACTATTCAAGTCCAAACGGTCAAATTCGTCCTTTTTTCAGGAGACGATCCAATGCGCCTCACCATTCGAATTAATGGCAGCGAATCGGCAACCCGGCAATCCTTCGCGGTACTGTGG<u>GTG</u>
P10	<u>TGA</u>CAACCGGCTGCGCATTCCTCTGACAATTTCCAAGATGCTGGCGCTCTTTATAAGCAAAAGCGCCCGGTTTTTCAATCCCGTCGATCAAGAAACCGTTACCACGTCCTAAATGGCGCTTTTGCGCCGCCCAACCTGTAAGAGTTACCAGTTACCGCCCCCTCGTGCCGCGCGATGTAATGCATGCATACAAAAGCACAAATCCGAGGACACC<u>ATG</u>
P14	<u>TGA</u>TTTTCCGCACGCCGGCGCCGGCCGCGGCCGTTTCATACGGCCGCGCCAGTTTTTTCCATTGCGACCCGCAATTCATCCGATCGTTGATTTTGTCATTAATAATCCCGGAAACGATTTCATAATAAATCCGGAAAAATCGATTACATAAGTGCCTGCTCCATTTTCAATGATCGCCGGTTTTCTAATAAACGGAATAGTGAATTGGTAAATGTTGAGCAAGGTCACGATGCGCTATGCTGTAAAAAGGTAAAAAGAAAATGCGGGACAGAGTGCCGCGGTATTTTTTTCTCTGCGATGTCGGCGCATCGCAGGCTTGGGCGGCCGCTCGAAATGAAATAAGGGTTTACACCTAGCATTAATGCCATTTTATTGACAATTAATCGATCTAGAATGACATCCTCCAACTTCCCGTACTCAAATGAGTGGCGGGTTGTCAATAAGTAAGCCGGCTTTTGATCTGGCTTCTATTTTTCTTTACGTCGTCTCTCGCATTGCCAACAGGAGCGTGCCCG<u>ATG</u>
P15	<u>CAT</u>GCGGACGGTTCCAAAAACGGCGCCCCCAGGCGGGGCGCCCGAAAAAGTTTCAAGGTGAAAAAGCTGCGCGCGACGAACGACGATGGCGCAGATGACGTAGCAGACTAATCAGGGACAATTCAGCCGGTGTGCCCCGCGCGGATTCGCTCCGTCACGGTGCGCAGCGCCCGTTATCTGTTTATAATCAAAGCAATTTTAAAGGATTCGAAGGTGGTTGT<u>ATG</u>
P17	<u>TAA</u>CCCGCAGCAGCGAACCCGTCACATGATGGAGCAGCCGGCGGCCTCTCGGCCCCGGCCCAAGGTTTAGGAGCAAGAACGCCTGATGCAAAGCATGCCCGGTGCCTTAGCGGCCCAACGACACGACAGGCACTTTGCGAACCCTTCAGTGGGATGGGCAAACTTCCCTCCGGATAAAGCGGACGGCCGTGTGGCGGTCCGCTTGATCAAGCGATTAGGGGTAGCGGCGCGAGGTGCGCCGGCTACCGACTTGATTTGGCGCGCGAGGCCATGTGGTCACGCGCGCCATACCGTATTCATTAGCGTAAGCGCGCGGTATCCGCGTACGCCGATGAATAGCCCGCTTCGAAGCGCACGTCCTGAACAGCGTCCGCGGGAAGCGGGTTTTGACGAAGGGTGTAGTTTGAACTGACTCTCATCTGAACCTGAAGGAGAACAAC<u>ATG</u>
P18	<u>TAA</u>CGACTTGATCGAACCCGTGGAACAGTAGCGACCGCCCCCGAATACGAAACGACGCGGGAAGGCACCGCTCGCCCATCGTTTCACGGCAACCCGGCGATGGGGGCACGTCGCGCACGGCTCCGCCCACCAAGAAACAAATTCCACCATTCCACATTAAAAGAAAACGTTATTCCATATCAAGCTGGATCACCGACCTATTTTGGATCCATTTTCCCTCCTGTCCCCCGTATCATTTCTCCCATTCAGAATCAGCAAGAAACGGAC<u>ATG</u>
P19	<u>TGA</u>CCCGCCCGCAAGCGCCGTCGCCGCCCGATTTGCCGCCCTTGTTCCTCGCACGCCGACCCGCGCGGGGCGCGTCGGCGCGCGGCCGCCGATCCGATCGGTCCGTTGGTGCGCAGCGACACCGCCACCCCGATTTGACGCTCTTTCACGCACGAACGAAAAGGGGTTGTAATCCGCTGTGATATAACTGCCGACGTGATCCGCTCCTTGAGCGAGACTCACTCCGAACGTCATCCGATGGAGAATAACGATGCAAAAACGGAATCTT<u>GTG</u>

The underlined letters are the initiation and termination codons.
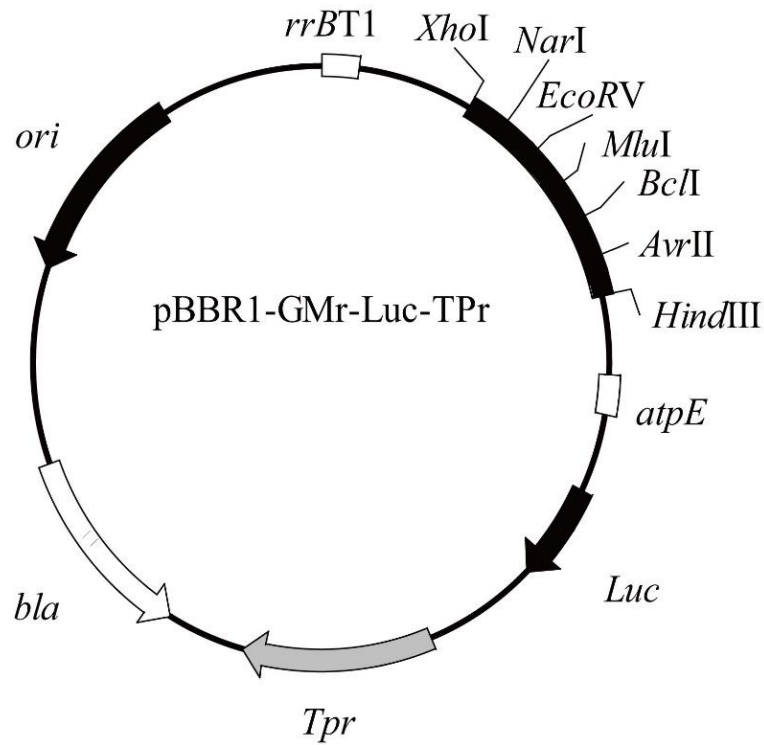
Figure S1. Trap vector with two reporter genes. The vector contains the Tp-resistant dihydrofolate reductase gene (*TP*r) and the luciferase gene set (*Luc*). The most relevant restriction sites that can be introduced into the promoter region are provided. *ori*, origin of replication; *bla*, gene encoding the ampicillin resistance (AmpR) protein; *atpE*, translation initiation region; *rrnBT1*, transcriptional terminator.
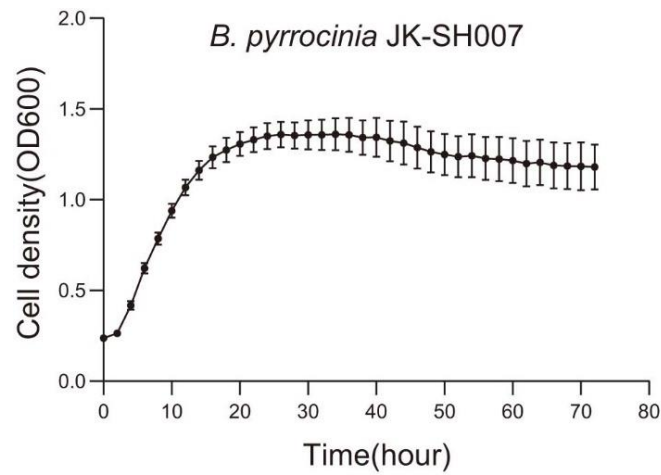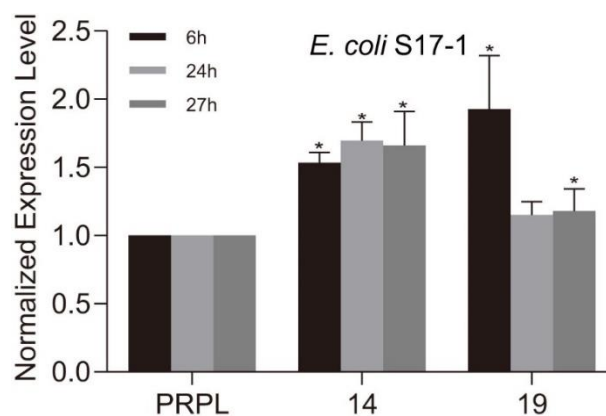

Figure S2. Growth curves of *B. pyrrocinia* JK-SH007. *B. pyrrocinia* JK-SH007 were cultured on LB medium. The standard deviations of the 10 independent replicates are indicated by the error bars.
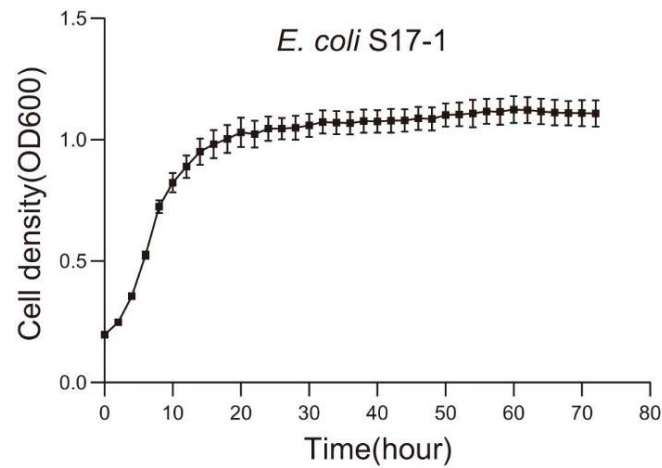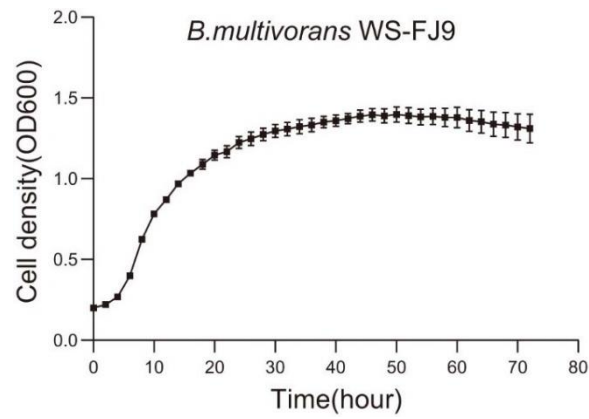
Figure S3. Growth curves of *E. coli* S17-1. *E. coli* S17-1were cultured on LB medium. The standard deviations of the 10 independent replicates are indicated by the error bars.
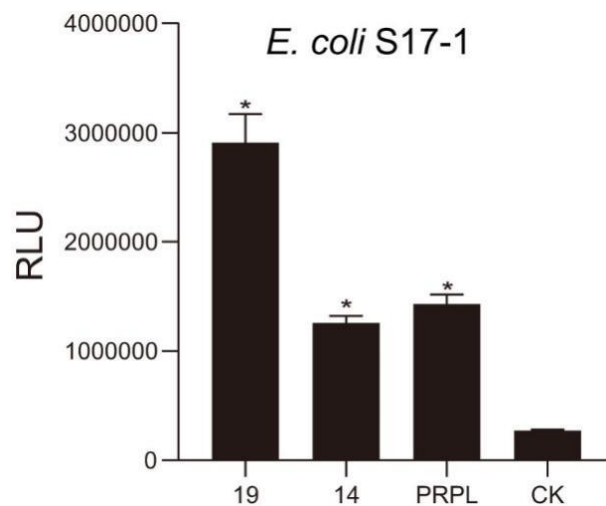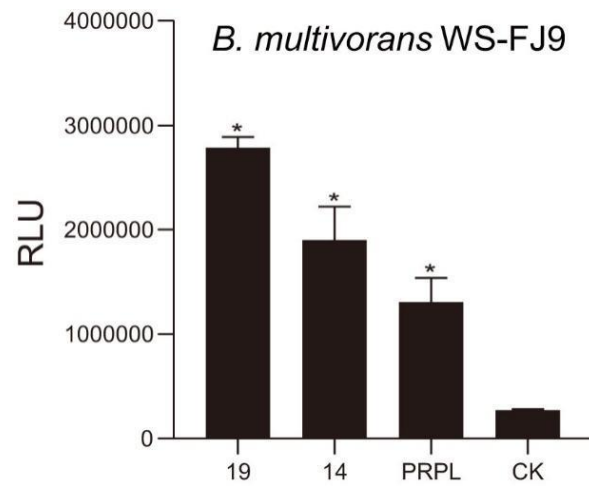

Figure S4. Assay of constitutive promoters from *E. coli* S17-1 using firefly luciferase. PRPL was used as the control variable. CK was a luciferase-specific positive control. The standard deviations from three separate replicates are indicated by the error bars. The data represent means ± SD ($n = 4$). * $p < 0.05$.
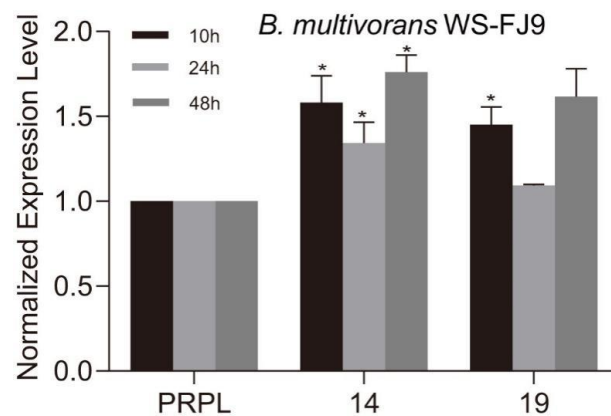

Figure S5. Promoter characterization via qPCR analysis in *E. coli* S17-1. In three different periods, the transcription of the firefly luciferase gene under strong promoters in JK-SH007 was measured. The y-axis depicts the expression value of PRPL, which was set to 1. The standard deviations from three separate replicates are indicated by the error bars. The data represent means ± SD ($n = 4$). * $p < 0.05$.

Figure S6. Growth curves of *B. multivorans* WS-FJ9. *B. multivorans* WS-FJ9 were cultured on LB medium. The standard deviations of the 10 independent replicates are indicated by the error bars.

Figure S7. Assay of constitutive promoters from *B. multivorans* WS-FJ9 using firefly luciferase. PRPL was used as the control variable. CK was a luciferase-specific positive control. The standard deviations from three separate replicates are indicated by the error bars. The data represent means ± SD ($n = 4$). * $p < 0.05$.

Figure S8. Promoter characterization via qPCR analysis in *B. multivorans* WS-FJ9. In three different periods, the transcription of the firefly luciferase gene under strong promoters in JK-SH007 was measured. The y-axis depicts the expression value of PRPL, which was set to 1. The standard deviations from three separate replicates are indicated by the error bars. The data represent means ± SD ($n = 4$). * $p < 0.05$.

References

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2. Tomlin, K.L.; Clark, S.R.; Ceri, H. Green and red fluorescent protein vectors for use in biofilm studies of the intrinsically resistant Burkholderia cepacia complex. *J. Microbiol. Methods* **2004**, *57*, 95–106, https://doi.org/10.1016/j.mimet.2003.12.007.
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