iScience, Volume *24*

Supplemental information

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A newly CcpA binding site plays a bidirectional role in carbon catabolism in *Bacillus licheniformis*

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Legends:

Transparent Methods

Bacterial Strains, Plasmids, and Culture Conditions

Table S1 lists the strains and plasmids used or produced in this study. *Escherichia coli* JM109 was used for plasmid preparation. The *E. coli* JM109 transformants carrying plasmids based on pHY-PLK300, were selected on Luria–Bertani (LB) agar plates supplemented with ampicillin (100 μg/mL). The *B. licheniformis* B1391 transformants were selected on LB agar plates supplemented with tetracycline (20 μg/mL). Two strains were cultured in LB medium (10 g/L tryptone, 5 g/L yeast extract, 10 g/L NaCl), *E. coli* was cultured at 37 °C and 200 rpm, and *B. licheniformis* was cultured at 37 °C and 250 rpm. Fermentation medium (12 g/L tryptone, 24 g/L yeast extract, 16.427 g/L $K_2HPO_4.3H_2O$, 2.31 g/L KH_2PO_4) was prepared for protein expression. A 3% seed liquid was inoculated into the fermentation medium, and batch fermentation was cultured at 37 °C and 250 rpm.

Plasmid Construction

The recombinant plasmids were constructed based on pHY300-PLK using the primers listed in Table S2. First, eGFP was amplified by the primer pair eGFP-F/eGFP-R (Table S2). The fragment was then purified and digested with *Xho*I and *Sma*I, followed by incorporation into pHY300-PLK, yielding pE. Then, the promoter region, P*treA*, was cloned using 100 ng of the genome of *Bacillus licheniformis* CICIM B1391. PCR was performed with high-fidelity DNA polymerase (Vazyme Biotech Co., Ltd. 2 × Phanta Master Mix) and the primer pairs P*treA*-F/P*treA*-R. The fragment was also purified and digested with *Hin*dIII and *Xho*I, followed by incorporation into pE, yielding pBLTE. Next, the artificial promoter P*treA* was constructed using overlap extension PCR. Two fragments, P*treA*-CRETre-1 and P*treA*-CRETre-2, were cloned by the primer pairs P*treA*-F/ P*treA*-CRETre-R, P*treA*-CRETre-F/ P*treA*-R, using P*treA* as template. P*treA*-CRETre was created using P*treA*-CRETre-1 and P*treA*-CRETre-2 as a template, and P*treA*-F/P*treA*-R as primers. The fragment P*treA*-CRETre was also purified and digested with *Hin*dIII and *Xho*I, followed by incorporation into pE, yielding pBLT1E. pBLT2E was constructed according to the construction method of pBLT1E.

Transformation of *B. licheniformis*

A method of electrotransformation was used to transform plasmids into *B. licheniformis*. A total of 8 μg-10 μg of plasmid was added to the *B. licheniformis* competent cells and mixed. Then, the mixed competent cells were added to a precooled 0.1 cm Gene Pulser cuvette and placed on ice for 5 min. Next, the 0.1 cm Gene Pulser cuvette was placed into an electroporation apparatus and shocked with 2,100 V. After the electric shock was administered, 900 μL of recovery medium (LB + 0.5 M sorbitol + 0.38 M mannitol) was immediately added, and the cells were cultured at 37°C and 100 rpm for 3 h before being applied to the corresponding antibiotic plates.

Fluorescence Measurement of eGFP

The BlspTE strain was cultured overnight at 37°C and 250 rpm, and 3% culture was inoculated into the fermentation broth. After 8 h of growth, 15 g/L trehalose was added for inducible expression. The sample was measured for OD600 and fluorescence intensity after 16 h of adding trehalose. Then, 100 μL of fermentation broth was centrifuged to obtain the cell pellet, then the cell pellet was rinsed twice with phosphate buffered saline (PBS) solution at pH 7.4, and the final OD600 was diluted to 0.5. Next, 200 μL of diluted suspension was added to the 96-well microtiter plate (Corning). The 96-well microtiter plate was placed in a TECAN-SparK plate reader (Tecan, Männedorf, Switzerland), which calculated the final value using an absorption wavelength of 485 nm, excitation wavelength of 535 nm, and gain value of 100. The formula FI (AU/OD) = 2×(FVt-FVr), where FVt refers to the fluorescence value measured by the target strain, and FVr represents the fluorescence value measured by the control strain BlsPE, was used to evaluate the fluorescence intensity.

Electrophoretic Mobility Shift Assays

DNA probes were amplified using high-fidelity DNA polymerase (Vazyme Biotech Co., Ltd. 2 × Phanta Master Mix) and primers were labeled with biotin. Biotin-labeled probes were purified by agarose gel electrophoresis. 10 nM biotin-labeled probes were incubated with different concentrations of CcpA in binding buffer (10 mM Tris-HCl (pH 7.4), 1 mM DTT, 1 mM EDTA, 50 mM KCl, 0.05 μg/μL poly (dI-dC), 1 mM $MgCl_2$), and the reaction solution was put through a full reaction at 25°C for 20 min. After the reaction was completed, the samples were separated by electrophoresis using 4% acrylamide gels in 0.5 × Tris-borate EDTA (TBE) buffer. The samples were electroblotted from the acrylamide gels onto nylon membrane (Beyotime, FFN15), and immobilized by UV crosslinking. The nylon membrane was washed and detected using Chemiluminescent EMSA Kit (Beyotime, GS009) according to manufacturing protocol. Gel imaging and analysis were performed using ChemiDoc XRS (Bio-Rad, U.S.A).

Statistical analysis

The sample size was $n \geq 3$ for biology experiments. A student's tests ($^*P \leq 0.05$; $^{**}P \leq 0.01$, $^{***}P \leq 0.001$) were performed for statistical analysis.

Fig. S1

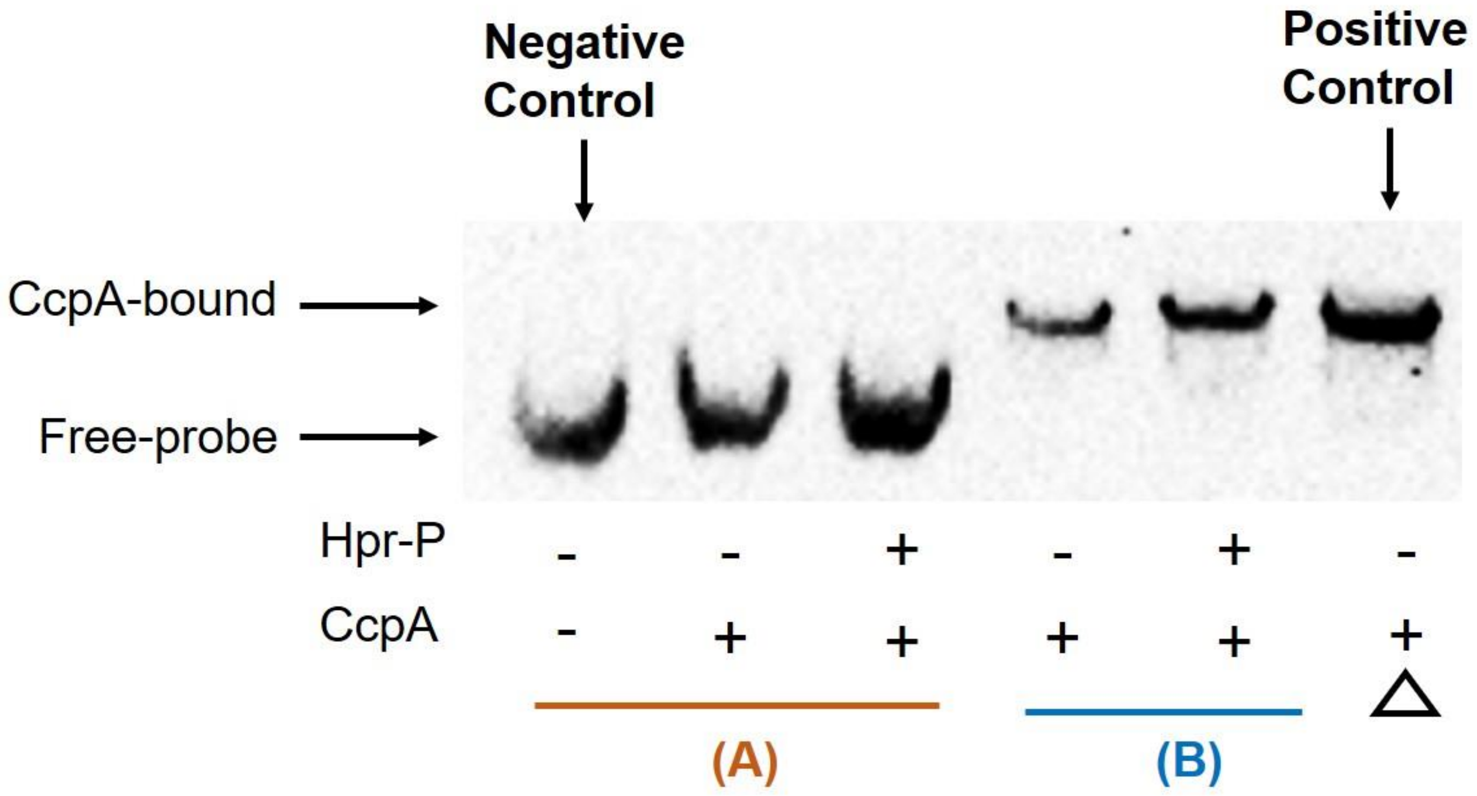


Figure S1: EMSA of CcpA or CcpA-Hpr-P protein for fragment A and B. Related to Figure 1.

The *hpr* (c16360) gene of *Bacillus licheniformis* CICIM B1391 was cloned and then inserted into pET28a vector (between *Nde*I and *Eco*RI), to obtain the gene expression vector of pET28a-*hpr*. The recombinant strain *E.coli* BL21 (DE3) containing expression vector was grown overnight in 15 mL LB-Kanamycin at 37℃, 200 rpm. A 3 % of the culture was inoculated into Terrific Broth (TB) at 37℃ until OD600 of 0.4-0.5. The Hpr protein was induced by 0.1mM IPTG for 10 h at 25℃. The cells were collected by centrifugation at 8,000 × g for 5 min. The Hpr protein was purified by the Kit (Mag-Beads His-Tag Protein Purification, Sangon Biotech, C650033) according to the manufacture's protocols. The purify of the Hpr Protein was analyzed through Tris-Tricine SDS PAGE.

Hpr protein phosphorylation was performed in 20 mM Tris-Cl buffer (pH 7.0) supplemented with 1 mM $MgCl_2$, 2 mM NaCl and 5 mM ATP. 27 µg Hpr protein and 0.15 µg HprK protein was added to the reaction system at 37℃ for 10 min. The reaction was ended at 75℃ for 5 min. 10% phos-tag SDS PAGE (Boppard, 193-16711) was used to determine whether Hpr phosphorylation.

The EMSA Lane (left→right): 10 nM fragment A; 1.2µM CcpA+10 nM fragment A; 1.2 µM CcpA +1.2 µM Hpr-P+10 nM fragment A; 1.2µM CcpA+10 nM fragment B; 1.2 µM CcpA +1.2 µM Hpr-P+10 nM fragment B; 1.2µM CcpA + 10 nM Positive control probe.

Fig. S2

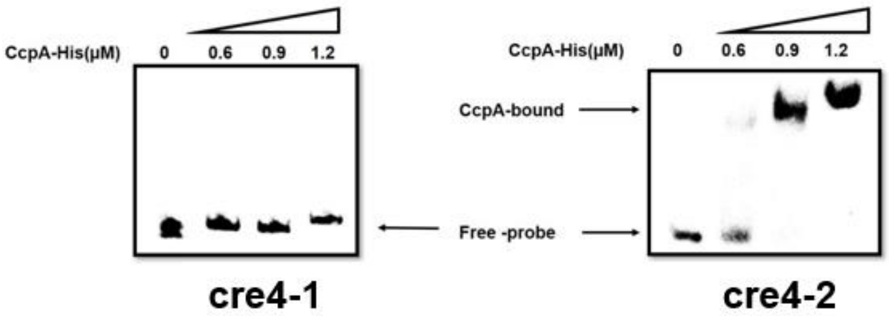
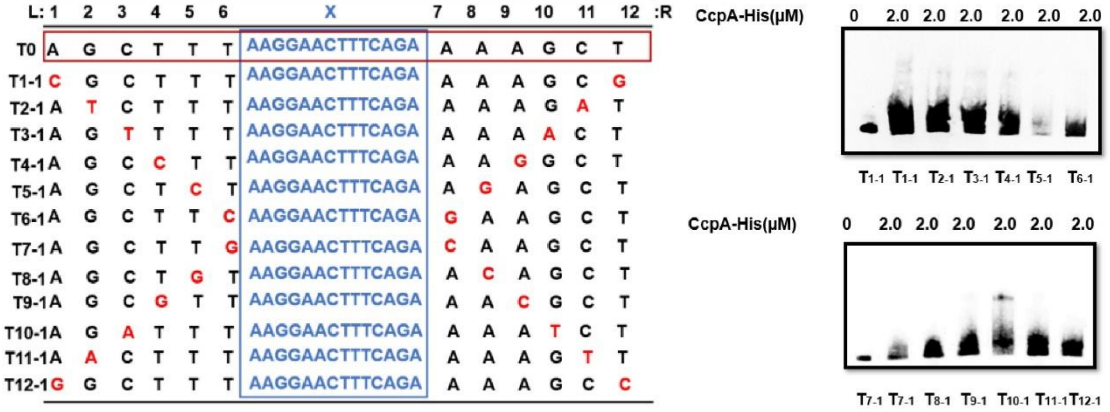


Figure S2: EMSA of CcpA protein binding to two fragments (E6, E7) labelled with 5’-biotin. Related to Figure 1.

Fragment E6 and Fragment E7, containing cre4-1 (AGCGTT-aaggaactttcaga-AACGCT) and cre4-2 (AGCTTT-aaggaactttcaga-AAAGCT). Increasing concentrations of CcpA (0 μM, 0.6 μM, 0.9 μM, 1.2 μM) were incubated with 10 nM E6 or E7 before the reaction run on EMSA gel.

Fig. S3

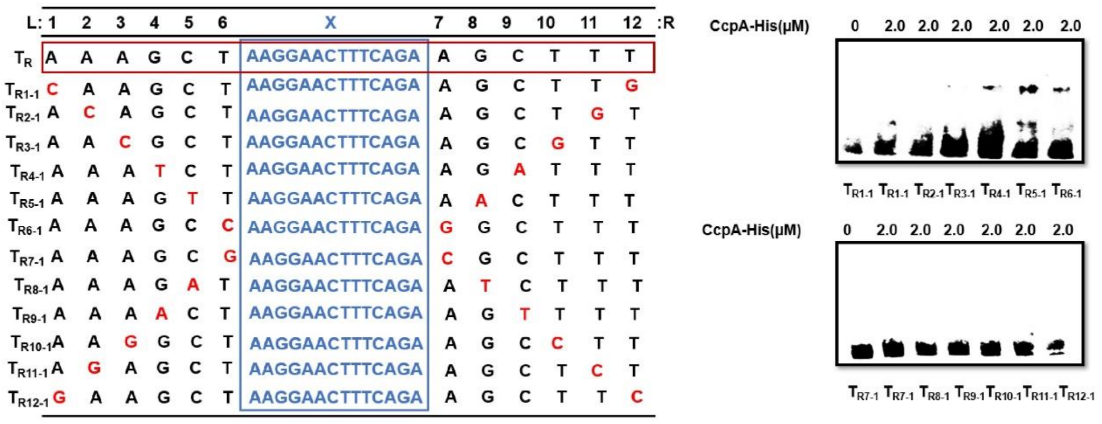


	L: 1	2	3	4	5	6	X	7	8	9	10	11	12 :R
T0	A	G	C	T	T	T	AAGGAACTTTCAGA	A	A	A	G	C	T
T1-1	C	G	C	T	T	T	AAGGAACTTTCAGA	A	A	A	G	C	G
T2-1	A	T	C	T	T	T	AAGGAACTTTCAGA	A	A	A	G	A	T
T3-1	A	G	T	T	T	T	AAGGAACTTTCAGA	A	A	A	A	C	T
T4-1	A	G	C	C	T	T	AAGGAACTTTCAGA	A	A	G	G	C	T
T5-1	A	G	C	T	C	T	AAGGAACTTTCAGA	A	G	A	G	C	T
T6-1	A	G	C	T	T	C	AAGGAACTTTCAGA	G	A	A	G	C	T
T7-1	A	G	C	T	T	G	AAGGAACTTTCAGA	C	A	A	G	C	T
T8-1	A	G	C	T	G	T	AAGGAACTTTCAGA	A	C	A	G	C	T
T9-1	A	G	C	G	T	T	AAGGAACTTTCAGA	A	A	C	G	C	T
T10-1	A	G	A	T	T	T	AAGGAACTTTCAGA	A	A	A	T	C	T
T11-1	A	A	C	T	T	T	AAGGAACTTTCAGA	A	A	A	G	T	T
T12-1	G	G	C	T	T	T	AAGGAACTTTCAGA	A	A	A	G	C	C

Figure S3: Nucleotide mutations within the 12-bp symmetrical regions of $\mathrm{CRE}_{\mathrm{Tre}}$. Related to Figure 3.

Two symmetrical bases in symmetrical regions were mutated based on fragment H1, resulting 12 derivative probes (T1-1, T2-1, T3-1, T4-1, T5-1, T6-1, T7-1, T8-1, T9-1, T10-1, T11-1, and T12-1). 2.0 μM CcpA was incubated with 10 nM T1-1, T2-1, T3-1, T4-1, T5-1, T6-1, T7-1, T8-1, T9-1, T10-1, T11-1, and T12-1before the reaction run on EMSA gel.

Fig. S4



	L: 1	2	3	4	5	6	X	7	8	9	10	11	12 :R
T_R	A	A	A	G	C	T	AAGGAACTTTCAGA	A	G	C	T	T	T
T_{R1-1}	C	A	A	G	C	T	AAGGAACTTTCAGA	A	G	C	T	T	G
T_{R2-1}	A	C	A	G	C	T	AAGGAACTTTCAGA	A	G	C	T	G	T
T_{R3-1}	A	A	C	G	C	T	AAGGAACTTTCAGA	A	G	C	G	T	T
T_{R4-1}	A	A	A	T	C	T	AAGGAACTTTCAGA	A	G	A	T	T	T
T_{R5-1}	A	A	A	G	T	T	AAGGAACTTTCAGA	A	A	C	T	T	T
T_{R6-1}	A	A	A	G	C	C	AAGGAACTTTCAGA	G	G	C	T	T	T
T_{R7-1}	A	A	A	G	C	G	AAGGAACTTTCAGA	C	G	C	T	T	T
T_{R8-1}	A	A	A	G	A	T	AAGGAACTTTCAGA	A	T	C	T	T	T
T_{R9-1}	A	A	A	A	C	T	AAGGAACTTTCAGA	A	G	T	T	T	T
T_{R10-1}	A	A	G	G	C	T	AAGGAACTTTCAGA	A	G	C	C	T	T
T_{R11-1}	A	G	A	G	C	T	AAGGAACTTTCAGA	A	G	C	T	C	T
T_{R12-1}	G	A	A	G	C	T	AAGGAACTTTCAGA	A	G	C	T	T	C

Figure S4: Further point mutation of the 12-bp symmetrical region of $\mathrm{CRE}_{\mathrm{Tre(R)}}$. Related to Figure 6.

Two symmetrical bases in symmetrical regions were mutated based on fragment H1, resulting 12 derivative probes (TR1-1, TR2-1, TR3-1, TR4-1, TR5-1, TR6-1, TR7-1, TR8-1, TR9-1, TR10-1, TR11-1, and TR12-1). 2.0 μM CcpA was incubated with 10 nM (TR1-1, TR2-1, TR3-1, TR4-1, TR5-1, TR6-1, TR7-1, TR8-1, TR9-1, TR10-1, TR11-1, and TR12-1), and T12-1before the reaction run on EMSA gel.

Fig. S5

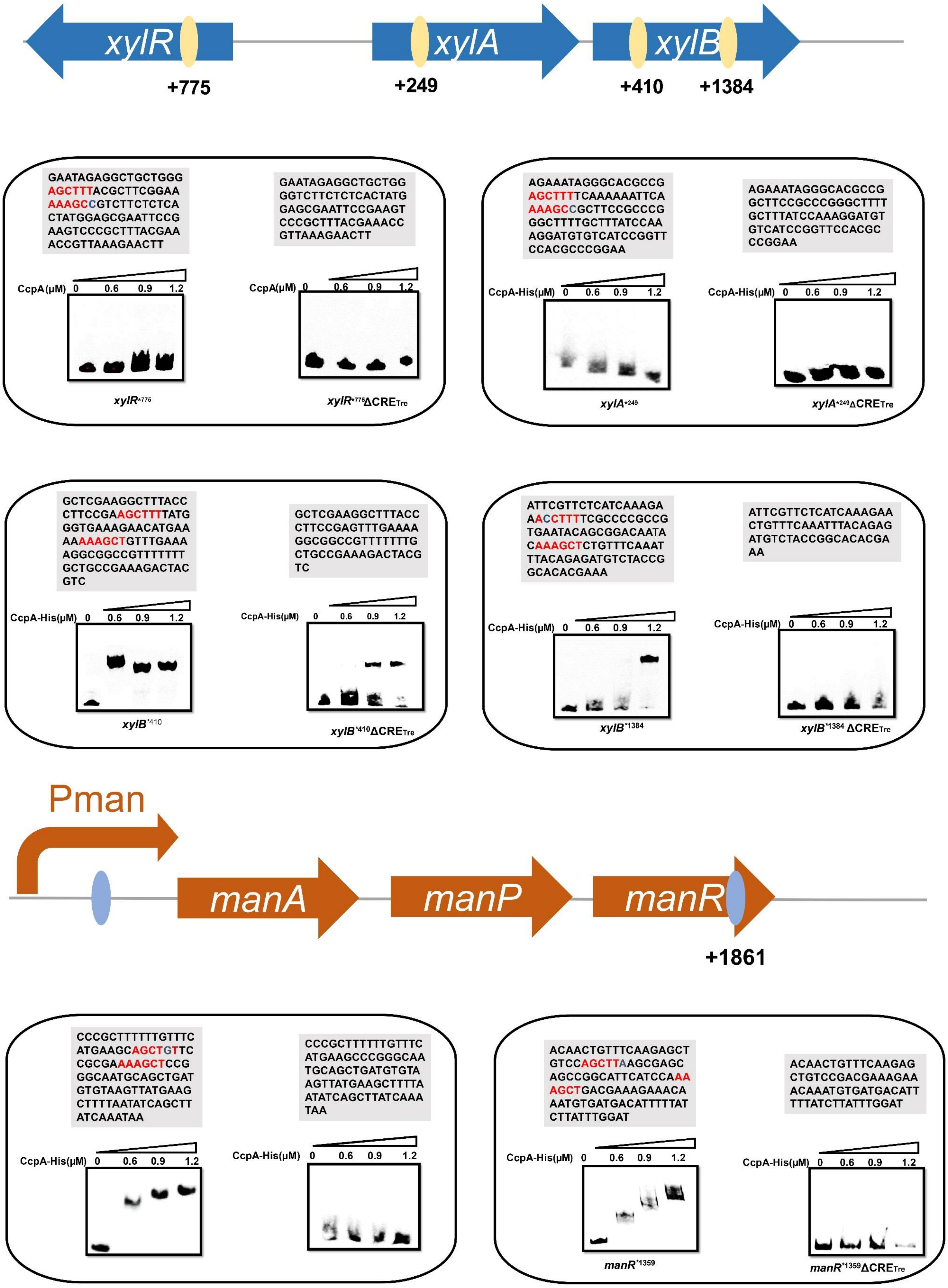


Figure S5: Potential CRE$_{Tre}$ sites in xylose operon and mannose operon. Related to Figure 8.

The putative CRE$_{Tre}$ was annotated with red. Increasing concentrations of CcpA (0 μM, 0.6 μM, 0.9 μM, 1.2 μM) were incubated with 10 nM fragments before the reaction run on EMSA gel.

Table S1

Bacterial Strains and Plasmids Used in This Study. Related to Figure 4.

Strain or plasmid	Description	Reference
Strains		
*Escherichia coli*JM109	F′, traD36, proAB + lacIq, Δ(lacZ), M15/Δ (lac-proAB), gln V44, e14−, gyrA96, recA1, relA1, endA1, thi, hsdR17 (CICIM B0012)	CICIM-CU
Bacillus licheniformis CICIM B1391	wild-type (CICIM B1391)	CICIM-CU
Bacillus licheniformis CA	*B. licheniformis* CICIM B1391, Δ*ccpA*	Laboratory construct
BlspHY	*B. licheniformis* CICIM B1391, harboring pHY300-PLK	this work
BlspE	*B. licheniformis* CICIM B1391, harboring pE	this work
BlspTE	*B. licheniformis* CICIM B1391, harboring pBLTE	this work
BlspT1E	*B. licheniformis* CICIM B1391, harboring pBLT1E	this work
BlspT2E	*B. licheniformis* CICIM B1391, harboring pBLT2E	this work
BlspDE	*B. licheniformis* CICIM B1391, harboring pBLDE	this work
BlspAE	*B. licheniformis* CICIM B1391, harboring pBLAE	this work
BlspHY1	*B. licheniformis* CA, harboring pHY300-PLK	this work
BlspE1	*B. licheniformis* CA, harboring pE	this work
BlspTE1	*B. licheniformis* CA, harboring pBLTE	this work
BlspT1E1	*B. licheniformis* CA, harboring pBLT1E	this work
BlspT2E1	*B. licheniformis* CA, harboring pBLT2E	this work
BlspDE1	*B. licheniformis* CA, harboring pBLDE	this work
BlspAE1	*B. licheniformis* CA, harboring pBLAE	this work
Plasmids		this work
pMD18-T-simple	*E. coli* cloning vector, Ap^{R}	TaKaRa
pHY300-PLK	*E. coli*/*Bacillus* shuttle vector, Ap^{R}/Tet^{R}	TaKaRa
pE	pHY300-PLK derivative with egfp	this work
pBLTE	pE derivative with P*treA* from *B. licheniformis* CICIM B1391	this work
pBLT1E	pE derivative with P*treA,* in which CRE site 'TGAAAGCGCTATAA' was changed to 'AGCTTT-AT-AAAGCT'	this work
pBLT2E	pE derivative with P*treA,* in which CRE site 'TGAAAGCGCTATAA' was changed to 'AAAGCT-AT-AGCTTT'	this work
pBLDE	pE derivative with P*dnaP* from *B. licheniformis* CICIM B1391	this work
pBLAE	pE derivative with P5A2 from *B. licheniformis* CICIM B1391	this work

Ap^{R}, ampicillin resistance; Tet^{R}, tetracycline resistance; Kan^{R}, kanamycin resistance

CICIM-CU: Culture and Information Center of Industrial Microorganisms of China Universities.

Table S2
Primers Used to Construct Recombinant Plasmids. Related to Figure 4.

Primers	Sequence (5’-3’)	Restriction site
eGFPF	CCGCTCGAGatgggtcgcggatccatg	*Xho*I
eGFPR	TCCCCCGGGtcacacgtggtggtggtg	*Sma*I
P*treA*-F	CCCAAGCTTatctcagccggttgttcc	*Hin*dIII
P*treA*-R	CCGCTCGAGttccaatccctccttctc	*Xho*I
P*treA*-CRETre-F	AGCTTTATAAAGCTaaatatgttgactacttgt	
P*treA*-CRETre-R	AGCTTTATAAAGCTaaaataaaaaaagcccgg	
P*treA*-CRETre(R)-F	AAAGCTATAGCTTTaaatatgttgactacttgt	
P*treA*-CRETre(R)-R	AAAGCTATAGCTTTaaaataaaaaaagcccgg	
P*danP*-F	CCCAAGCTTacgcttagggtagcgaatcga	*Hin*dIII
P*dnaP*-R	CCGCTCGAGcggcctgaacactctatata	*Xho*I
P5A2-F	CCCAAGCTTagggaggggaaagctaaat	*Hin*dIII
P5A2-R	CCGCTCGAGctaggattttacctccctttc	*Xho*I
treR-199-F	atgaagatcaacaagtatat	
treR-169-F	aaacaaattgcagaacaaat	
treR-139-F	atactgaatgccggagata	
treR-109-F	gaaaacgaccttgccgaac	
treR-79-F	tcgagggaaacggttcgga	
treR-49-F	gtgcttgcccagaacggat	
treR-199-R(5'-biotin)	ctgacccttttcctttgatct	
treR-202-F	agatcaaaggaaaagggt	
treR-172-F	tcgtgctgcacagggaga	
treR-142-F	cggtttccggtttggtcag	
treR-112-F	tttcagaaacgcttggcaa	
treR-82-F	cgacggtccacgagttcgg	
treR-52-F	cggatgcatatattcaaaa	
treR-202-R(5'-biotin)	acgacccgccatacctctt	
treR-196-F	ttctggagatgaagaggtatg	
treR-196-R(5'-biotin)	gaccacaatttccttatg	
treR-194-F	atgcccataaggaaattg	
treR-194-R(5'-biotin)	ctacttttctcttcttgca	
P*treA*-88-F	atctcagccggttgttcccggc	
P*treA*-88-R(5'-biotin)	aaataaaaaaagcccggccg	
P*treA*-102-F	ttgaaagcgctataaaaat	
P*treA*-102-R(5'-biotin)	ttccaatccctccttctc	
PtreA-102ΔCRE-F	taaatatgttgactacttg	
tre-26-1-F	AGCTTTaaggaactttcagaAAAGCTatctcagccggttgttcccg	
tre-20-2-F	AGCTTTaaggttcagaAAAGCTatctcagccggttgttcccg	
tre-12-3-F	AGCTTTAAAGCTatctcagccggttgttcccg	
tre-26-4-F	CCCGGGaaggaactttcagaAAAGCTatctcagccggttgttcccg	
tre-26-5-F	AGCTTTaaggaactttcagaCCCGGGatctcagccggttgttcccg	
tre-26-6-F	CCCGGGaaggaactttcagaCCCGGGatctcagccggttgttcccg	
tre(R)-26-1-F	AAAGCTaaggaactttcagaAGCTTTatctcagccggttgttcccg	
tre(R)-20-2-F	AAAGCTaaggttcagaAGCTTTatctcagccggttgttcccg	
tre(R)-12-3-F	AAAGCTAGCTTTatctcagccggttgttcccg	
tre(R)-26-4-F	CCCGGGaaggaactttcagaAGCTTTatctcagccggttgttcccg	
tre(R)-26-5-F	AAAGCTaaggaactttcagaCCCGGGatctcagccggttgttcccg	
tre(R)-26-6-F	CCCGGGaaggaactttcagaCCCGGGatctcagccggttgttcccg	
T1-F	CGCTTTaaggaactttcagaAAAGCTatctcagccggttgttcccg	
T2-F	ATCTTTaaggaactttcagaAAAGCTatctcagccggttgttcccg	
T3-F	AGTTTTaaggaactttcagaAAAGCTatctcagccggttgttcccg	
T4-F	AGCCTTaaggaactttcagaAAAGCTatctcagccggttgttcccg	
T5-F	AGCTCTaaggaactttcagaAAAGCTatctcagccggttgttcccg	
T6-F	AGCTTCaaggaactttcagaAAAGCTatctcagccggttgttcccg	
T7-F	AGCTTTaaggaactttcagaCAAGCTatctcagccggttgttcccg	
T8-F	AGCTTTaaggaactttcagaACAGCTatctcagccggttgttcccg	
T9-F	AGCTTTaaggaactttcagaAACGCTatctcagccggttgttcccg	
T10-F	AGCTTTaaggaactttcagaAAATCTatctcagccggttgttcccg	
T11-F	AGCTTTaaggaactttcagaAAAGTTatctcagccggttgttcccg	
T12-F	AGCTTTaaggaactttcagaAAAGCCatctcagccggttgttcccg	
TR-1-F	CAAGCTaaggaactttcagaAGCTTTatctcagccggttgttcccg	
TR-2-F	ACAGCTaaggaactttcagaAGCTTTatctcagccggttgttcccg	
TR-3-F	AACGCTaaggaactttcagaAGCTTTatctcagccggttgttcccg	
TR-4-F	AAATCTaaggaactttcagaAGCTTTatctcagccggttgttcccg	

TR-5-F	AAAGTTaaggaactttcagaAGCTTTatctcagccggttgttcccg
TR-6-F	AAAGCCaaggaactttcagaAGCTTTatctcagccggttgttcccg
TR-7-F	AAAGCTaaggaactttcagaCGCTTTatctcagccggttgttcccg
TR-8-F	AAAGCTaaggaactttcagaATCTTTatctcagccggttgttcccg
TR-9-F	AAAGCTaaggaactttcagaAGTTTTatctcagccggttgttcccg
TR-10-F	AAAGCTaaggaactttcagaAGCCTTatctcagccggttgttcccg
TR-11-F	AAAGCTaaggaactttcagaAGCTCTatctcagccggttgttcccg
TR-12-F	AAAGCTaaggaactttcagaAGCTTCatctcagccggttgttcccg
T1-1-F	CGCTTTaaggaactttcagaAAAGCGatctcagccggttgttcccg
T2-1-F	ATCTTTaaggaactttcagaAAAGATatctcagccggttgttcccg
T3-1-F	AGTTTTaaggaactttcagaAAAACTatctcagccggttgttcccg
T4-1-F	AGCCTTaaggaactttcagaAAGGCTatctcagccggttgttcccg
T5-1-F	AGCTCTaaggaactttcagaAGAGCTatctcagccggttgttcccg
T6-1-F	AGCTTCaaggaactttcagaGAAGCTatctcagccggttgttcccg
T7-1-F	AGCTTGaaggaactttcagaCAAGCTatctcagccggttgttcccg
T8-1-F	AGCTGTaaggaactttcagaACAGCTatctcagccggttgttcccg
T9-1-F	AGCGTTaaggaactttcagaAACGCTatctcagccggttgttcccg
T10-1-F	AGATTTaaggaactttcagaAAATCTatctcagccggttgttcccg
T11-1-F	AACTTTaaggaactttcagaAAAGTTatctcagccggttgttcccg
T12-1-F	GGCTTTaaggaactttcagaAAAGCCatctcagccggttgttcccg
TR-1-1-F	CAAGCTaaggaactttcagaAGCTTGatctcagccggttgttcccg
TR-2-1-F	ACAGCTaaggaactttcagaAGCTGTatctcagccggttgttcccg
TR-3-1-F	AACGCTaaggaactttcagaAGCGTTatctcagccggttgttcccg
TR-4-1-F	AAATCTaaggaactttcagaAGATTTatctcagccggttgttcccg
TR-5-1-F	AAAGTTaaggaactttcagaAACTTTatctcagccggttgttcccg
TR-6-1-F	AAAGCCaaggaactttcagaGGCTTTatctcagccggttgttcccg
TR-7-1-F	AAAGCGaaggaactttcagaCGCTTTatctcagccggttgttcccg
TR-8-1-F	AAAGATaaggaactttcagaATCTTTatctcagccggttgttcccg
TR-9-1-F	AAAACTaaggaactttcagaAGTTTTatctcagccggttgttcccg
TR-10-1-F	AAGGCTaaggaactttcagaAGCCTTatctcagccggttgttcccg
TR-11-1-F	AGAGCTaaggaactttcagaAGCTCTatctcagccggttgttcccg
TR-12-1-F	GAAGCTaaggaactttcagaAGCTTCatctcagccggttgttcccg
xylB-410-F	agcttttatgggaaagaaca
xylB-410ΔCRETre-F	gtttgaaaaggcggccgt
xylB-410-R(5'-biotin)	attcgctgtgaagacgccc
xylB-1384-F	accttttcgccccgccgtga
xylB-1384ΔCRETre-F	ctgtttcaaatttacagaga
xylB-1384-R(5'-biotin)	ttattcccgaaagctcgcca
xylR-775-F	agctttacgcttcggaaaaagc
xylR-775ΔCRETre-F	gtcttctctcactatggag
xylR-775-R(5'-biotin)	gaaaccgttaaagaacttgc
xylA-249-F	agcttttcaaaaaattca
xylA-249ΔCRETre-F	gcttccgcccgggctttt
xylA-249-R(5'-biotin)	atgacacatcctttggataaagc
levR-1359-F	agcttttttaatcagaaaat
levR-1359ΔCRETre-F	tcaaaaccacagcttgctca
levR-1359-R(5'-biotin)	aattaaaggaagtcgctgaa
manR-1359-F	agcttttggatgaatgccgg
manR-1359ΔCRETre-F	ggacagctcttgaaacagt
manR-1359-R(5'-biotin)	ctttatgctggctgtcaaac
Ptre--48-F	cgctttcaaaaataaaaaaa
Ptre--71-F	cggccgttcccttcataa
Ptre--48-R(5'-biotin)	atctcagccggttgttcccg