SUPPLEMENTARY INFORMATION

Transcriptional regulation by BglJ-RcsB, a pleiotropic heteromeric transcriptional activator in *Escherichia coli*

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The supplementary information includes Tables S1 (*E. coli* K12 strains), S2 (Plasmids), S3 (Oligonucleotides), and supplementary references (52,53).

Table S1	<i>E. coli</i> K12 strains	
Strain	relevant genotype ^a	Reference / Construction ^a
BW30270	= MG1655 rph^+	Datsenko and Wanner,
	Laboratory collection No S3839	deposited at the Coli
		Genetic Stock Center
		#7925
S1734	<i>bglJ_c</i> (constitutive <i>bglJ</i> expression, allele <i>yjjQ/bglJ</i> -Y6::mTn10-cmR)	(31)
BRE2055	BRE2047 Δ <i>crp96 zhd-732</i> ::Tn10	(52)
S541	Δ bgl Δ lacZ ara thi	(26)
S3010	S541 Δ <i>hns</i> ::kanR _{KD4}	(4)
S3910	S541 <i>bglJ_c</i> (cmR)	S541 x T4 <i>GT7</i> (S1734)
S3974	BW30270 <i>ilvG</i> ⁺ (not motile)	(4)
S4197	S3974 $\Delta lacZ$	(4)
T314	S4197 ΔleuO _{FRT} Δ(yjjP-yjjQ-bglJ) _{FRT}	(20)
T1032	S4197 $\Delta leuO_{FRT} bglJ_c$ (cmR)	(20)
T15	S4197 <i>ArcsB</i> ::kanR _{KD4}	(4)
Т23	S4197 Δ(<i>yjjP-yjjQ-bglJ</i>) _{FRT}	(20)
T75	S3974 Δ(<i>yjjP-yjjQ-bglJ</i>) _{FRT}	(20)
T1030	S4197 $bgIJ_c$ (cmR, BgI ⁺)	S4197 x T4 <i>GT7</i> (S1734)
T1048	S3974 Δ(<i>yjjP-yjjQ-bglJ</i>) _{FRT} Δhns _{FRT} stpA::Tet ^κ	(20)
T1166	S3974 <i>bglJ_c</i> (cmR, Bgl ⁺)	x T4 <i>GT7</i> (S1734)
Strains with	insertions of promoter <i>lacZ</i> fusions at <i>attB</i> (derivatives of T314)	
T1188	attB::[PchiA lacZ spec ^k]	T314/pLDR8 x pKESL9
T1478	attB::[<i>PchiA lacZ</i> spec ^{κ}] <i>bglJ_c</i> (cmR)	T1188 x T4GT7 (S1734)
T1772	attB::[<i>PchiA lαcZ</i> spec [*]] <i>bglJ_c</i> (cmR) <i>ΔrcsB</i> ::kanR _{KD4}	T1478 x T4GT7 (T15)
T1191	attB::[<i>PmolR lacZ</i> spec [°]]	T314/pLDR8 x pKESL3
T1441	attB::[<i>PmolR lacZ</i> spec ۗ] <i>bglJ_c</i> (cmR)	T1191 x T4GT7 (S1734)
T1773	attB::[<i>PmolR lacZ</i> spec [^]] bglJ _c (cmR) ΔrcsB::kanR _{KD4}	T1441 x T4GT7 (T15)
T1226	attB::[PmolR _{mut} lacZ spec]	T314/pLDR8 x pKESL13
T1444	attB::[<i>PmolR_{mut} lacZ</i> spec [~]] <i>bglJ_c</i> (cmR)	T1226 x T4GT7 (S1734)
T1181	attB::[<i>PsfsB lacZ</i> spec [*]]	T314/pLDR8 x pKESL5
T1436	attB::[<i>PsfsB lacZ</i> spec [*]] <i>bglJ_c</i> (cmR)	T1181 x T4GT7 (S1734)
T1774	attB::[<i>PsfsB lacZ</i> spec [^]] bglJ _c (cmR) ΔrcsB::kanR _{KD4}	T1436 x T4GT7 (T15)
T1228	attB::[PsfsB _{mut} lacZ spec [¬]]	T314/pLDR8 x pKESL15
T1446	attB::[<i>PsfsB_{mut} lacZ</i> spec ^K] <i>bglJ_c</i> (cmR)	T1228 x T4GT7 (S1734)
T1186	attB::[<i>PyecT lacZ</i> spec [°]]	T314/pLDR8 x pKESL8
T1476	attB::[<i>PyecT lacZ</i> spec] <i>bglJ_c</i> (cmR)	T1186 x T4GT7 (S1734)
T1775	attB::[<i>PyecT lacZ</i> spec [°]] <i>bglJ_c</i> (cmR) Δ <i>rcsB</i> ::kanR _{KD4}	T1476 x T4GT7 (T15)
T1189	attB::[<i>PygiZ lacZ</i> spec [°]]	T314/pLDR8 x pKESL10
T1438	attB::[<i>PygiZ lacZ</i> spec] <i>bglJ_c</i> (cmR)	T1189 x T4GT7 (S1734)
T1776	attB::[<i>PygiZ lacZ</i> spec [°]] $bgIJ_c$ (cmR) $\Delta rcsB$::kanR _{KD4}	T1438 x T4GT7 (T15)
T1236	attB::[PygiZ _{mut} lacZ spec [*]]	T314/pLDR8 x pKESL20
T1450	attB::[<i>PygiZ_{mut} lacZ</i> spec``] <i>bglJ_c</i> (cmR)	T1236 x T4GT7 (S1734)

Table S1	E. coli K12 strains	
Strain	relevant genotype ^a	Reference / Construction ^a
T1184	attB::[<i>PyidL lacZ</i> spec ^R]	T314/pLDR8 x pKESL7
T1474	attB::[<i>PyidL lacZ</i> spec ^R] <i>bglJ_c</i> (cmR)	T1184 x T4GT7 (S1734)
T1777	attB::[<i>PyidL lαcZ</i> spec ^R] <i>bglJ_c</i> (cmR) Δ <i>rcsB</i> ::kanR _{KD4}	T1474 x T4GT7 (T15)
T1579	attB::[<i>PyidL_{mut} lacZ</i> spec [®]]	T314/pLDR8 x pKESL92
T1581	attB::[<i>PyidL_{mut} lacZ</i> spec ^R] <i>bglJ_c</i> (cmR)	T314/pLDR8 x pKESL92
T1177	attB::[<i>PykiA lacZ</i> spec [®]]	T314/pLDR8 x pKESL1
T1472	attB::[<i>PykiA lacZ</i> spec ^R] <i>bglJ_c</i> (cmR)	T1177 x T4GT7 (S1734)
T1778	attB::[<i>PykiA lacZ</i> spec [®]] <i>bglJ_c</i> (cmR) <i>∆rcsB</i> ::kanR _{KD4}	T1472 x T4GT7 (T15)
T1552	attB::[PykiA _{mut} lacZ spec ^R]	T314/pLDR8 x pKESL41
T1557	attB::[<i>PykiA_{mut} lacZ</i> spec ^R] <i>bglJ_c</i> (cmR)	T314/pLDR8 x pKESL41
T1179	attB::[<i>PynbA lacZ</i> spec ^R]	T314/pLDR8 x pKESL2
T1326	attB::[<i>PynbA lacZ</i> spec ^R] <i>bglJ_c</i> (cmR)	T1179 x T4GT7 (S1734)
T1779	attB::[<i>PynbA lacZ</i> spec ^R] <i>bglJ_c</i> (cmR) <i>∆rcsB</i> ::kanR _{KD4}	T1326 x T4GT7 (T15)
T1224	attB::[<i>PynbA_{mut} lacZ</i> spec ^R]	T314/pLDR8 x pKESL12
T1442	attB::[<i>PynbA_{mut} lacZ</i> spec ^R] <i>bglJ_c</i> (cmR)	T1224x T4GT7 (S1734)
T1183	attB::[<i>PynjI lacZ</i> spec ^R]	T314/pLDR8 x pKESL6
T1327	attB::[<i>PynjI lacZ</i> spec ^R] <i>bglJ_c</i> (cmR)	T1183 x T4GT7 (S1734)
T1780	attB::[<i>PynjI lacZ</i> spec ^R] <i>bglJ_c</i> (cmR) <i>∆rcsB</i> ::kanR _{KD4}	T1327 x T4GT7 (T15)
T1230	attB::[<i>PynjI_{mut} lacZ</i> spec ^R]	T314/pLDR8 x pKESL16
T1448	attB::[<i>PynjI_{mut} lacZ</i> spec ^R] <i>bglJ_c</i> (cmR)	T1230 x T4GT7 (S1734)
T1180	attB::[<i>PyqhG lacZ</i> spec ^R]	T314/pLDR8 x pKESL4
T1473	attB::[<i>PyqhG lacZ</i> spec ^R] <i>bglJ_c</i> (cmR)	T1180 x T4GT7 (S1734)
T1781	attB::[<i>PyqhG lacZ</i> spec ^R] <i>bglJ_c</i> (cmR) <i>∆rcsB</i> ::kanR _{KD4}	T1473 x T4GT7 (T15)
T568	attB::[<i>PbgI</i> t1 _{RAT} <i>bgIG lacZ</i> spec ^R]	(4)
T1432	T568 <i>bglJ_c</i> (cmR)	T568 x T4GT7 (S1734)
T1500	T568 Δ <i>crp96 zhd-732</i> ::Tn10	T568 x T4GT7 (BRE2055)
T1502	T568 Δ <i>crp96-zhd-732</i> ::Tn10 <i>bglJ_c</i> (cmR)	T1432 x T4GT7 (BRE2055)
T729	T568 hns _{FRT}	(4)
T580	attB::[<i>PbgI_</i> BgIJ-RcsB _{mut1} t1 _{RAT} <i>bgIG lacZ</i> spec ^R]	(4)
T1434	T580 <i>bglJ_c</i> (cmR)	T580 x T4GT7 (S1734)
T1501	T580 Δ <i>crp96 zhd-732</i> ::Tn10	T580 x T4GT7 (BRE2055)
T1503	T580 <i>bglJ_c</i> (cmR) Δ <i>crp96 zhd-</i> 732::Tn10	T1434 x T4GT7 (BRE2055)
T735	T580 hns _{FRT}	(4)
T1413	attB::[<i>PbgI_</i> CRP _{mut} t1 _{RAT} <i>bgIG lacZ</i> spec ^R]	T314/pLDR8 x pKESL46
T1507	T1413 <i>bglJ_c</i> (cmR)	T1413 x T4GT7 (S1734)
T1504	T1413 Δ <i>crp</i> 96 <i>zhd-732</i> ::Tn10	T1413 x T4GT7 (BRE2055)
T1550	T1413 <i>bglJ_c</i> (cmR) Δ <i>crp96 zhd-732</i> ::Tn10	T1507 x T4GT7 (BRE2055)
T1543	T1413 Δ <i>hns</i> ::kan _{KD4}	T1413 x T4GT7 (S3010)
T1465	attB::[Pbgl_BglJ-RcsB _{mut1} _CRP _{mut} t1 _{RAT} bglG lacZ spec ^R]	T314/pLDR8 x pKESL47
T1493	T1465 <i>bglJ_c</i> (cmR)	T1465 x T4GT7 (S1734)
T1506	T1465 Δ <i>crp96 zhd-732</i> ::Tn10	T1465 x T4GT7 (BRE2055)
T1530	T1465 <i>bglJ_c</i> (cmR) Δ <i>crp96 zhd-732</i> ::Tn10	T1493 x T4GT7 (BRE2055)
T1545	T1465 Δ <i>hns</i> ::kan _{κD4}	T1465 x T4GT7 (S3010)

^a Strain construction was performed as described previously (15,26). Bacteriophage T4*GT7* (28) was used for transduction with the donor strains given in brackets. Transductants of the $bgIJ_c$ (cmR) allele were selected on LB chloramphenicol plates and analyzed by PCR. Co-transduction of $\Delta crp96$ with zhd-732::Tn10 was screened on MacConkey Maltose plates, and Maltose negative colonies were taken. Integration of reporter constructs into the phage λ *attB* site was performed using origin-less re-ligated BamHI fragments of the plasmids, which carry a promoter *lacZ* fusion, the *attP*-site for integration, and the *aadA* gene for selection on LB spectinomycin plates. These orgin-less circular DNA molecules were used to transform strain T314 carrying the *int* expressing helper-plasmid pLDR8 (25,26).

Table S2	Plasmids	
Plasmid	Features ^{a, b}	Reference / Construction ^{c, d, e}
pDCRP	<i>crp</i> in pBR-ori ampR	(35)
pDCRP-H159L	crp-H159L (CAC->CTG) in pBR-ori ampR	(35)
pDCRP-H19L	crp-H19L (CAC->CTC) in pBR-ori ampR	this work ^c
(=pKES324)		
pDCRP-K101E	<i>crp</i> -K101E (AAA->GAA) in pBR-ori ampR	this work ^c
(=pKES328)		
pDctrl	vector control for pDCRP (= pBR-ori ampR), sequence at	pDCRP BamHI, Sall, re-ligated
(=pKES325)	BamHI, Sall fusion is GAATTCCCGGGGATCcgaccgatgcccttga	
pFMAC20	<i>E-Pbgl t1 bglG lacZ</i> p15A-ori kanR attP specR	(53)
pFMAC19	pFMAC20 with CAATTG insertion at -61,5 relative to P2bgl	(53)
pKES209	pFMAC20 with CAATTG insertion at -69,5 relative to P2bgl	this work ^d
pKES203	pFMAC20 with CAATTG insertion at -80,5 relative to P2bgl	this work ^d
pKES205	pFMAC20 with CAATTG insertion at -84,5 relative to P2bgl	this work ^d
pKES207	pFMAC20 with CAATTG insertion at -88,5 relative to P2bgl	this work ^d
pLDR8	cI_{857} P _R λ - <i>int</i> in pSC101 rep ^{ts} kanR	(25)
pKES268	PlacUV5 MCS lacZ p15A-ori kanR attP specR	this work ^e
pKESL9	PchiA (pos:3468190-3467882) lacZ	cloned PCR fragment T672, T673
pKESL3	PmolR(yehH) (pos:2194338-2194505) lacZ	cloned PCR fragment T658, T659
pKESL13	PmolR _{mut} (TTCTTT aga AAAT ag CTAAAG) lacZ	T699 for mutagenesis
pKES329	PmolR 5bp insertion lacZ	this work ^f
pKES330	PmolR 10bp insertion lacZ	this work ^f
pKESL5	PsfsB (pos:3332698-3352942) lacZ	cloned PCR fragment T662, T663
pKESL15	PsfsB _{mut} (ATCTTT ga ATAT ag TGAATA) <i>lacZ</i>	T701 for mutagenesis
pKESL8	PyecT (pos:1959790-1960001) lacZ	cloned PCR fragment T676, T677
pKESL4	<i>PyqhG</i> (pos:3155455-3155686) <i>lacZ</i>	cloned PCR fragment T660, T661
pKESL10	<i>PygiZ</i> (pos:3170219-3170566) <i>lacZ</i>	cloned PCR fragment T674, T675
pKESL20	PygiZ _{mut} (GGATAAgaATTTagTGAAAA) lacZ	T705 for mutagenesis
pKESL7	<i>PyidL</i> (pos:3858054-3878320) <i>lacZ</i>	cloned PCR fragment T668, T669
pKESL92	PyidL _{mut} (GCAGTCAGATagTTAAAATA) lacZ	T816 for mutagenesis
pKES331	PyidL 5bp insertion lacZ	this work ^f
pKES332	PyidL 10bp insertion lacZ	this work ^f
pKESL1	<i>PykiA</i> (pos:0407683-0407892) <i>lacZ</i>	cloned PCR fragment T654, T655
pKESL41	PykiA _{mut} (GGAGGGCATTTT ag TGAAAT) <i>lacZ</i>	T765 and T812 for mutagenesis
pKESL2	PynbA (pos:1475436-1475662) lacZ	cloned PCR fragment T656, T657
pKESL12	PynbA _{mut1} (ACCTTG ga ATAT ag TGAATT) <i>lαcZ</i>	T698 for mutagenesis
pKESL6	Pynjl (pos:1843066-1842886) lacZ	cloned PCR fragment T664, T665
pKESL16	РупјІ _{тиt1} (TTCGAG ga ATAT ag TGAAAT) <i>lacZ</i>	T702 for mutagenesis
pKENV61	Pbgl t1 _{RAT} bglG lacZ	(50)
pKES222	Pbgl_BglJ-RcsB _{mut1} (AACTTTATAAATagCTAAAAT) t1 _{RAT} bglG lacZ	(4)
pKESL46	Pbgl_CRP _{mut} (AACacCGACGATCCTCATTTT) t1 _{RAT} bglG lacZ	primer T770 for mutagenesis amplified from template pKFNV61
pKESL47	Pbgl_BglJ-RcsB _{mut1} CRP _{mut} t1 _{RAT} bglG lacZ	primer T770 for mutagenesis amplified from template pKES222

^a Unless otherwise stated the vector backbone of the plasmids carry a p15A derived origin of replication, the *neo* gene conferring kanamycin resistance (kanR), the *aadA* gene conferring spectinomycin resistance (specR), and the phage λ attP site, as described (26).

^b Positions in brackets refer to the reference genome sequence NC_000913 of *E. coli* K12 strain MG1655. Sequences in bracket refer to presumptive BgIJ-RcsB binding sites with site-specific mutations shown in lower case bold letters.

^c Plasmids expressing *crp* mutants were constructed by cloning overlapping PCR fragments into the Sall, BamHI digested pDcrp plasmid DNA. The PCR fragments were generated using oligonucleotides T870 and T871 for mutagenesis of residue 19, oligonucleotides T940 and T941 for mutagenesis of residue 101, oligonucleotides T24 and S527 for amplification, and pDCRP as template.

^d Plasmids carrying 6bp CAATTG insertions within the *bgl* regulatory region were generated by Munl digestion of plasmids carrying insertions of *lacO* (53). *E-Pbgl* carries a 1 bp exchange from A to G at position -79/-99 relative to the transcription start sites of *P2bgl* and *Pbgl* generating an EcoRI-site (53).

^e Plasmid pKES268 was used as cloning vector, pKES268 carries a *lacUV5* promoter fragment flanked by a Sall restriction site (upstream) and a multiple cloning site (MCS) including a Xbal site (downstream). The Sall and Xbal sites were used for cloning of promoter fragments amplified by PCR using cells of strain BW30270 as template.

^f The derivatives of *PmolR* and *PyidL lacZ* fusions with 5bp and 10 bp duplications in between the BglJ-RcsB binding site and the promoter were constructed by overlapping PCR using oligonucleotides T932 and T933 (*PmolR* +5bp), T934 and T935 (*PmolR* +10bp), T936 and T937 (*PyidL* +5bp), and T938 and T939 (*PyidL* +10bp).

Table S	Table S3 Oligonucleotides			
No	Sequence ^a	Purpose		
S527	AAGAATTCGCCGGCTTCCATTCAGGT	crp cloning, maps in		
		vector backbone		
T24	GAAAAGTGCCACCTGACGTCTAA	crp cloning, maps in		
		vector backbone		
T265	GCGC <u>GAATTC</u> CTGTAGAACGA	5'RACE EcoRI		
T268	AUAUGCGCGAAUUCCUGUAGAACGAACACUAGAAGAAA	5'RACE RNA adapter		
T654	gtcagtcgacTAATTCCTCGCCTTCCCCTTG	PykiA cloning		
T655	gtca <u>tctaga</u> TGCACGCCACCATTCATCAT	PykiA cloning		
T656	gtcagtcgacCGGGAACAAGCGATGAGAAAA	PynbA cloning		
T657	gtca <u>tctaga</u> TATCTGGTAGAGCGTCATTATCAATCCT	PynbA cloning		
T658	gtcagtcgacGGTCATCAGGTGAAATAATCCCCCC	PmolR cloning		
T659	gtca <u>tctaga</u> AGTGTCTCATTCTGCATCCCTGTAATT	PmolR cloning		
T660	gtcagtcgacGACCAGTTCGGCGGCTAACAT	PyqhG cloning		
T661	gtca <u>tctaga</u> AAGTATTATTTTCATCTGTTTTTCACCTCTCC	PyqhG cloning		
T662	gtcagtcgacCGTTAATCCCCTCCCTCATC	PsfsB cloning		
T663	gtca <u>tctaga</u> ATTACTTTCCATTTTTCCTCACTCCTTA	PsfsB cloning		
T664	gtcagtcgacAAAGTCCGCCAGCAGATGAAGTC	Pynjl cloning		
T665	gtca <u>tctaga</u> CTTTTTTCATCACATTCCCTGTTATTACATACT	Pynjl cloning		
T666	gtca <u>gtcgac</u> CGGGTGGGGGGATAATTCA	PyiaBA cloning		
T667	gtca <u>tctaga</u> CTTTGAGGTTTTCATAACGATCTCCATAT	PyiaBA cloning		
T668	gtcagtcgacGTATCTGTTTTGCGGACCTTCCAC	PyidL cloning		
T669	gtca <u>tctaga</u> CAATTTTCCATTCATTTTAATATCCCTCC	PyidL cloning		
T670	gtca <u>gtcgac</u> GACTTTCGCCTTTATTTGGGTGG	PyigGF cloning		
T671	gtca <u>tctaga</u> TCTGGAAATCTTACCGTTAGATGTTGG	PyigGF cloning		
T672	gtcagtcgacGTTGTTGCTAAAGTTCTGGGCTAATT	PchiA cloning		
T673	gtca <u>tctaga</u> CCCTTGTGACGTAAAAACTGCAAA	PchiA cloning		
T674	gtcagtcgacCAGGATGTTGCTCATTTTTAACCTC	PygiZ cloning		
T675	gtca <u>tctaga</u> TTTCTGCTTTAACATATATCAGTACGCTCAT	PygiZ cloning		
T676	gtca <u>gtcgac</u> CTGGGTATTGAGACTGTAGAGCGTATGTAA	PyecT cloning		
T677	gtca <u>tctaga</u> AAACACTGCGGTATTCCTTAAATTCA	PyecT cloning		
T698	5'phos TGAGTCCCCAATACCTTGgaATATagTGAATTTTTAATGAAACGG	PynbA mutagenesis		
T699	5'phos GACAGCGCCTTTTCTTT aga AAAT ag CTAAAGTTGTTTTCTTGC	PmolR mutagenesis		
T701	5'phos AATAGGGATGTGCATCTTTgaATATagTGAATATTCACACTCTTTACAG	PsfsB mutagenesis		
T702	5'phos TCTTTCGTGCTATTCGAGgaATATagTGAAATATCCAGCGGA	Pynjl mutagenesis		
T705	5'phos TCACCGATGCAGGATAAgaATTTagTGAAAATGACAATACTGATAG	PygiZ mutagenesis		
T707	gtca <u>tctaga</u> CGCCAACATCAACAGCAATC	ynbA 5-'RACE		
T708	gtca <u>tctaga</u> CCGAACAATAAGCCCCATCATAAT	ynjl 5'-RACE		
T709	gtca <u>tctaga</u> ATTGTATTCTTGTCGCAGGCATGA	<i>yecT</i> 5'-RACE		
T710	gtca <u>tctaga</u> CTTCTTCACCTTCTCCGCAATCAG	molR 5'-RACE		
T711	gtca <u>tctaga</u> CTGCGTGGTCTTTTCTTTATCATCTTT	yqhG 5'-RACE		
T712	gtca <u>tctaga</u> AAGCCACCAACCAAACCAG	<i>ygiZ</i> 5'-RACE		
T713	gtca <u>tctaga</u> GGCTTTCGCAATAATCATCTCTCC	sfsB 5'-RACE		

Table S3 Oligonucleotides			
No	Sequence	Purpose	
T714	gtca <u>tctaga</u> AATAGCCAGCGACCGATCCTG	ykiA 5'-RACE	
T715	gtca <u>tctaga</u> TGTAGAAGAAACCCACCAGGCATT	chiA 5'-RACE	
T716	gtca <u>tctaga</u> TGAGTTGGCGATAAGCGGTTT	yidL 5'-RACE	
T765	5'phos AACGGGAGGGCATTTT ag TGAAATATCCTTTCTTTAGCCCATAA	ykiA mutagenesis	
T770	5'phos CTATTGATAAAAATATGACCATGCTCGgtGTTATTAACTTTGTGTAATTTTAG	bgl CRP mutagenesis	
T812	5'phos TTATGGGCTAAAGAAAGGATATTTCA ct AAAATGCCCTCCCGTT	<i>ykiA</i> mutagenesis	
T816	5'phos TCGGGTCAGGCAGTCAGATagTTAAAATACAAACGTCGTATCC	yidL mutagenesis	
T820	GTTGTCGCTGTAATGTTTGTGTTCG	<i>ynjl,</i> qRT-PCR	
T821	CCGAACAATAAGCCCCATCATAA	<i>ynjl,</i> qRT-PCR	
T822	CATTATCTCTTGCCAGGCTTACGC	<i>yecT,</i> qRT-PCR	
T823	GAGATTGTATTCTTGTCGCAGGCA	<i>yecT,</i> qRT-PCR	
T828	TGGCATCGCTCAGGACATTCATC	<i>chiA</i> , qRT-PCR	
T829	CAGGATAAATGGAAGTCAGAAGGCAAAG	<i>chiA,</i> qRT-PCR	
T832	CGGTAACGGGCTCTCTCAAGGTAG	<i>molR</i> , qRT-PCR	
T833	CCAGCCACGGTAAAACAGCATC	<i>molR</i> , qRT-PCR	
T838	CATACCTACGCCGACGAGCATTAC	<i>yidL,</i> qRT-PCR	
T839	GCAGTGTTATTTGTTCGCCATTCTTTA	<i>yidL,</i> qRT-PCR	
T870	GGTACTTATGAATG a GGCAATGAGACAAGAACCATT	crp-H19L	
T871	CTTGTCTCATTGCCtCATCATAAGTACCCATCCAAGA	crp-H19L	
T932	AAAGTTGTT <u>ttctt</u> TTCTTGCGATTTTGTCTCTCTCTA	PmolR+5bp cloning	
T933	AATCGCaagaaAAGAAAACAACTTTAGGAATTTATAAAGAAAAGGC	PmolR+5bp cloning	
T934	AAGTTGTT <u>ttcttgcgat</u> TTCTTGCGATTTTGTCTCTCTCTA	PmolR+10bp cloning	
T935	CAAGAA <u>atcgcaagaa</u> AACAACTTTAGGAATTTATAAAGAAAAGGC	PmolR+10bp cloning	
T936	CTTAAAATA <u>caaac</u> CAAACGTCGTATCCCTGAACGGATT	PyidL+5bp cloning	
T937	GGATACGACgtttgGTTTGTATTTTAAGAATCTGACTGCCT	PyidL+5bp cloning	
T938	ACAAACgtcgacaaacGTCGTATCCCTGAACGGATT	PyidL+10bp cloning	
Т939	CGACgtttgtcgacGTTTGTATTTTAAGAATCTGACTGCCTG	PyidL+10bp cloning	
T940	ATTTCGTACAAAgAATTTCGCCAATTGATTCAG	<i>crp</i> -K101E	
T941	TTGGCGAAATTcTTTGTACGAAATTTCAGCCAC	<i>crp</i> -K101E	

^a Bases of the oligonucleotide which match the template are in capital letters, while other bases are in lower case letters. Mutations are indicated with lower case letters in bold. Restrictions sites are underlined. 5 and 10 bp sequence duplications (oligonucleotides T932-T939) are shown in lowercase and underlined letters.

SUPPLEMENTARY REFERENCES

- 52. Bremer, E., Gerlach, P. and Middendorf, A. (1988) Double negative and positive control of *tsx* expression in *Escherichia coli*. *J. Bacteriol.*, **170**, 108-116.
- Caramel, A. and Schnetz, K. (1998) Lac and Lambda repressor relieve silencing of the *Escherichia coli bgl* promoter. Activation by alteration of a repressing nucleoprotein complex. J. Mol. Biol., 284, 875-883.