

Supplementary Data

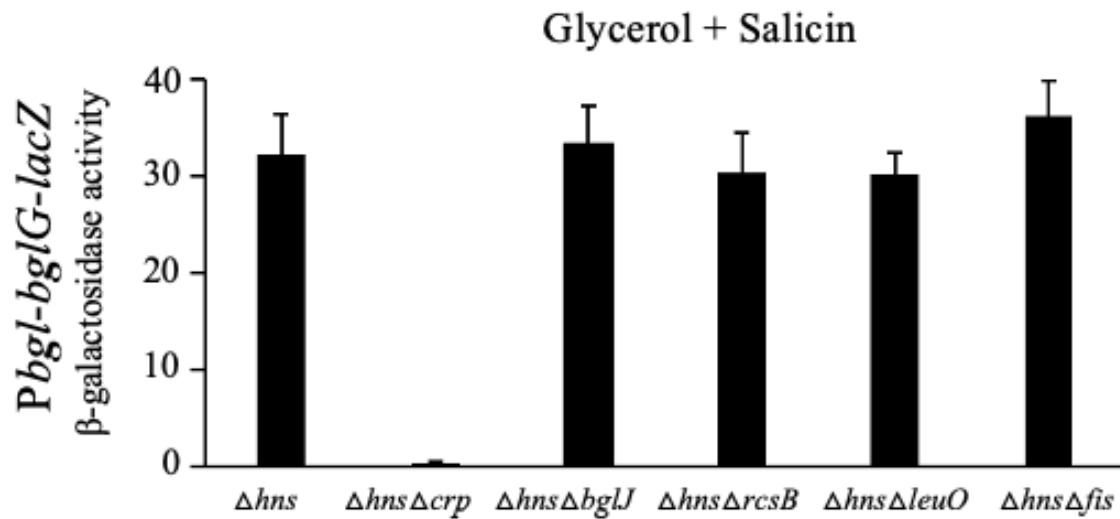


Figure S1. Effects of various transcription factors on *bgl* operon activities in the absence of H-NS. The genes encoding transcription regulators were individually deleted in a *hns* deletion background. Using the operon reporter *PbgI-bglG-lacZ*, the *bgl* operon activities from all the double mutants were measured. Strain cultures, sample collections and β-galactosidase assays were carried out as for Figure 1.

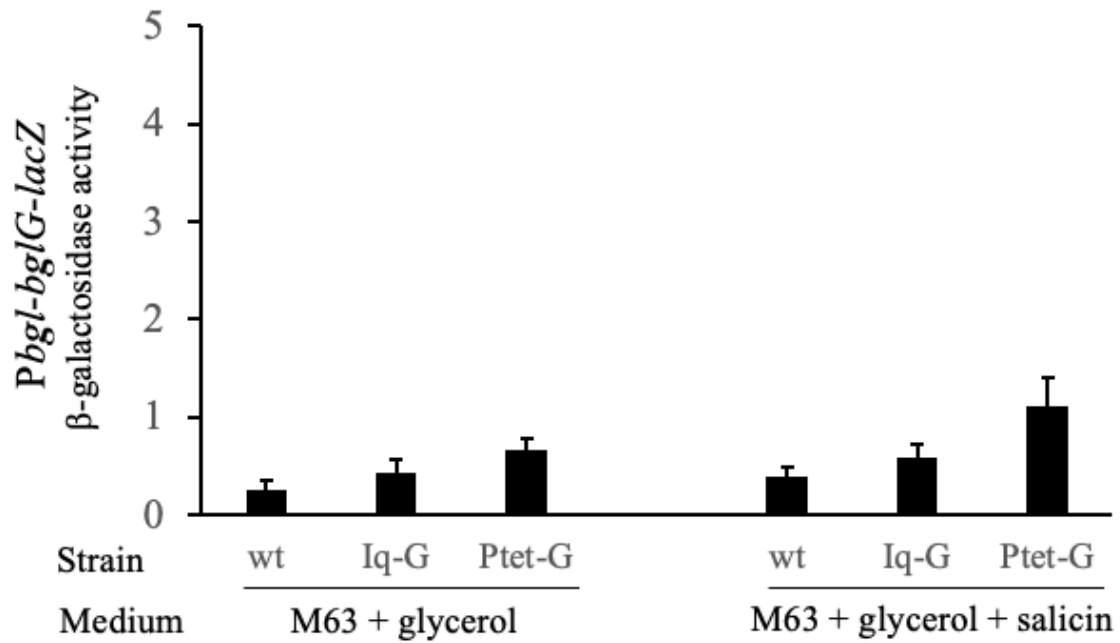


Figure S2. H-NS repression of *bgl* operon expression in cells over-expressing *bglG*. The *bglG* gene was driven by constitutive promoters *lacIq* (medium strength) and *Ptet* (strong) at the *intS* locus. Using *P_{bgl-bglG-lacZ}* as the reporter, the operon activities were measured when cells were cultured in M63 with glycerol only (left) or glycerol and salicin (right). Sample collection and β -galactosidase assays were carried out as for Figure 1.

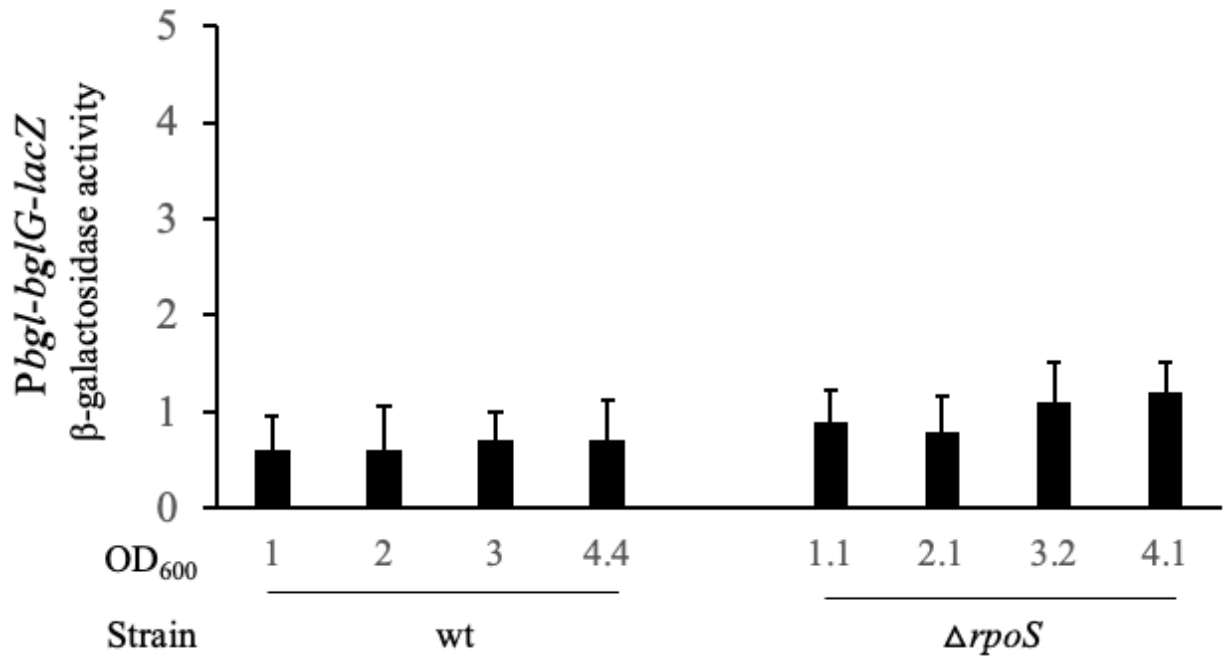


Figure S3. Effect of an *rpoS* deletion mutation on *bgl* operon expression as the cells approach and enter the stationary growth phase. Using *PbgI-bglG-lacZ* as the reporter, the operon activities were measured when wild type and $\Delta rpoS$ cells were cultured in M63 with glycerol and salicin at 37°C. Samples were collected when the OD₆₀₀ reached about 1, 2, 3 and 4, respectively. β-galactosidase assays were carried out as for Figure 1.

Supplementary Table S1. Strains and plasmids used in this study

Strains or plasmids	Genotype or description	Reference
Strains		
BW25113	Wild type, <i>lacI^q rrnB_{T14} ΔlacZ_{WJ} ΔhsdR514 ΔaraBAD_{AH33} ΔrhaBAD_{LD78}</i>	[1]
Bgl ⁺	IS5 in a reverse orientation at -207.5 upstream of <i>bglG</i> translation start site in BW25113	This study
Δ <i>crp</i>	Deletion of <i>crp</i> in BW25113	[2]
Δ <i>bglG</i>	Deletion of <i>bglG</i> in BW25113	This study
Δ <i>bglJ</i>	Deletion of <i>bglJ</i> in BW25113	This study
Δ <i>rcsB</i>	Deletion of <i>rcsB</i> in BW25113	This study
Δ <i>bglJ</i> Δ <i>rcsB</i>	Deletion of <i>bglJ</i> and <i>rcsB</i> in BW25113	This study
Δ <i>leuO</i>	Deletion of <i>leuO</i> in BW25113	This study
Δ <i>hns</i>	Deletion of <i>hns</i> in BW25113	This study
Δ <i>stpA</i>	Deletion of <i>stpA</i> in BW25113	This study
Δ <i>hns</i> Δ <i>stpA</i>	Deletion of <i>hns</i> and <i>stpA</i> in BW25113	This study
Δ <i>fis</i>	Deletion of <i>fis</i> in BW25113	This study
Iq-G	<i>lacIq</i> driving <i>bglG</i> at the <i>intS</i> locus in BW25113	[3]
Ptet-G	Ptet driving <i>bglG</i> at the <i>intS</i> locus in BW25113	[3]
BW_P <i>bgl</i> -Z	P <i>bgl</i> driving <i>lacZ</i> at the <i>lac</i> locus in BW25113	This study
Bgl ⁺ _P <i>bgl</i> -Z	P <i>bgl</i> driving <i>lacZ</i> at the <i>lac</i> locus in Bgl ⁺	This study
Δ <i>crp</i> _P <i>bgl</i> -Z	P <i>bgl</i> driving <i>lacZ</i> at the <i>lac</i> locus in Δ <i>crp</i>	This study
Δ <i>bglG</i> _P <i>bgl</i> -Z	P <i>bgl</i> driving <i>lacZ</i> at the <i>lac</i> locus in Δ <i>bglG</i>	This study
Δ <i>bglJ</i> _P <i>bgl</i> -Z	P <i>bgl</i> driving <i>lacZ</i> at the <i>lac</i> locus in Δ <i>bglJ</i>	This study
Δ <i>rcsB</i> _P <i>bgl</i> -Z	P <i>bgl</i> driving <i>lacZ</i> at the <i>lac</i> locus in Δ <i>rcsB</i>	This study
Δ <i>bglJ</i> Δ <i>rcsB</i> _P <i>bgl</i> -Z	P <i>bgl</i> driving <i>lacZ</i> at the <i>lac</i> locus in Δ <i>bglJ</i> Δ <i>rcsB</i>	This study
Δ <i>leuO</i> _P <i>bgl</i> -Z	P <i>bgl</i> driving <i>lacZ</i> at the <i>lac</i> locus in Δ <i>leuO</i>	This study
Δ <i>hns</i> _P <i>bgl</i> -Z	P <i>bgl</i> driving <i>lacZ</i> at the <i>lac</i> locus in Δ <i>hns</i>	This study
Δ <i>stpA</i> _P <i>bgl</i> -Z	P <i>bgl</i> driving <i>lacZ</i> at the <i>lac</i> locus in Δ <i>stpA</i>	This study
Δ <i>fis</i> _P <i>bgl</i> -Z	P <i>bgl</i> driving <i>lacZ</i> at the <i>lac</i> locus in Δ <i>fis</i>	This study
BW25113_G-Z	P <i>bgl</i> - <i>bglG</i> driving <i>lacZ</i> at the <i>lac</i> locus in BW25113	[3]
Bgl ⁺ _G-Z	P <i>bgl</i> - <i>bglG</i> driving <i>lacZ</i> at the <i>lac</i> locus in Bgl ⁺	This study
Δ <i>crp</i> _G-Z	P <i>bgl</i> - <i>bglG</i> driving <i>lacZ</i> at the <i>lac</i> locus in Δ <i>crp</i>	This study
Δ <i>bglG</i> _G-Z	P <i>bgl</i> - <i>bglG</i> driving <i>lacZ</i> at the <i>lac</i> locus in Δ <i>bglG</i>	This study
Δ <i>bglJ</i> _G-Z	P <i>bgl</i> - <i>bglG</i> driving <i>lacZ</i> at the <i>lac</i> locus in Δ <i>bglJ</i>	This study
Δ <i>rcsB</i> _G-Z	P <i>bgl</i> - <i>bglG</i> driving <i>lacZ</i> at the <i>lac</i> locus in Δ <i>rcsB</i>	This study
Δ <i>bglJ</i> Δ <i>rcsB</i> _G-Z	P <i>bgl</i> - <i>bglG</i> driving <i>lacZ</i> at the <i>lac</i> locus in Δ <i>bglJ</i> Δ <i>rcsB</i>	This study
Δ <i>leuO</i> _G-Z	P <i>bgl</i> - <i>bglG</i> driving <i>lacZ</i> at the <i>lac</i> locus in Δ <i>leuO</i>	This study
Δ <i>hns</i> _G-Z	P <i>bgl</i> - <i>bglG</i> driving <i>lacZ</i> at the <i>lac</i> locus in Δ <i>hns</i>	This study
Δ <i>stpA</i> _G-Z	P <i>bgl</i> - <i>bglG</i> driving <i>lacZ</i> at the <i>lac</i> locus in Δ <i>stpA</i>	This study
Δ <i>fis</i> _G-Z	P <i>bgl</i> - <i>bglG</i> driving <i>lacZ</i> at the <i>lac</i> locus in Δ <i>fis</i>	This study
Δ <i>hns</i> Δ <i>stpA</i> _G-Z	P <i>bgl</i> - <i>bglG</i> driving <i>lacZ</i> at the <i>lac</i> locus in Δ <i>hns</i> Δ <i>stpA</i>	This study
Ptet-G_G-Z	P <i>bgl</i> - <i>bglG</i> driving <i>lacZ</i> at the <i>lac</i> locus in Ptet-G	[3]
Iq-G_G-Z	P <i>bgl</i> - <i>bglG</i> driving <i>lacZ</i> at the <i>lac</i> locus in Iq-G	[3]
BW_IS5P <i>bgl</i> -Z	IS5P <i>bgl</i> driving <i>lacZ</i> at the <i>lac</i> locus in BW25113	This study
Δ <i>crp</i> _IS5P <i>bgl</i> -Z	IS5P <i>bgl</i> driving <i>lacZ</i> at the <i>lac</i> locus in Δ <i>crp</i>	This study
Δ <i>hns</i> _IS5P <i>bgl</i> -Z	IS5P <i>bgl</i> driving <i>lacZ</i> at the <i>lac</i> locus in Δ <i>hns</i>	This study
Δ <i>hns</i> Δ <i>stpA</i> _IS5P <i>bgl</i> -Z	IS5P <i>bgl</i> driving <i>lacZ</i> at the <i>lac</i> locus in Δ <i>hns</i> Δ <i>stpA</i>	This study
BW25113_IS5G-Z	IS5P <i>bgl</i> - <i>bglG</i> driving <i>lacZ</i> at the <i>lac</i> locus in BW25113	This study
Bgl ⁺ _IS5G-Z	IS5P <i>bgl</i> - <i>bglG</i> driving <i>lacZ</i> at the <i>lac</i> locus in Bgl ⁺	This study
Δ <i>crp</i> _IS5G-Z	IS5P <i>bgl</i> - <i>bglG</i> driving <i>lacZ</i> at the <i>lac</i> locus in Δ <i>crp</i>	This study
Δ <i>hns</i> _IS5G-Z	IS5P <i>bgl</i> - <i>bglG</i> driving <i>lacZ</i> at the <i>lac</i> locus in Δ <i>hns</i>	This study

Bgl ⁺ Δ <i>fis</i>	Deletion of <i>fis</i> in Bgl ⁺	This study
Bgl ⁺ Δ <i>crp</i>	Deletion of <i>crp</i> in Bgl ⁺	This study
Bgl ⁺ Δ <i>crp</i> Δ <i>fis</i>	Deletion of <i>crp</i> and <i>fis</i> in Bgl ⁺	This study
Bgl ⁺ Δ <i>fis</i> _IS5G-Z	IS5 <i>Pbgl-bglG</i> driving <i>lacZ</i> at the <i>lac</i> locus in Bgl ⁺ Δ <i>fis</i>	This study
Bgl ⁺ Δ <i>crp</i> _IS5G-Z	IS5 <i>Pbgl-bglG</i> driving <i>lacZ</i> at the <i>lac</i> locus in Bgl ⁺ Δ <i>crp</i>	This study
Bgl ⁺ Δ <i>crp</i> Δ <i>fis</i> _IS5G-Z	IS5 <i>Pbgl-bglG</i> driving <i>lacZ</i> at the <i>lac</i> locus in Bgl ⁺ Δ <i>crp</i> Δ <i>fis</i>	This study
<i>hns</i> L30P	Mutation of leucine to proline at 30 th codon in the <i>hns</i> gene	This study
<i>hns</i> L30P_ <i>Pbgl</i> -Z	<i>Pbgl</i> driving <i>lacZ</i> at the <i>lac</i> locus in <i>hns</i> L30P	This study
<i>hns</i> L30P_G-Z	<i>Pbgl-bglG</i> driving <i>lacZ</i> at the <i>lac</i> locus in <i>hns</i> L30P	This study
BW25113_tG-Z	A truncated <i>Pbgl-bglG</i> driving <i>lacZ</i> at the <i>lac</i> locus in BW25113	This study
Bgl ⁺ _tG-Z	A truncated <i>Pbgl-bglG</i> driving <i>lacZ</i> at the <i>lac</i> locus in Bgl ⁺	This study
Plasmids		
pKDT	A <i>rrnB</i> terminator (<i>rrnBT</i>) in pKD13	[4]
pKDT_ <i>Pbgl</i>	<i>Pbgl</i> cloned downstream of the <i>rrnBT</i> in pKDT	This study
pKDT_IS5 <i>Pbgl-bglG</i>	IS5 <i>Pbgl-bglG</i> cloned downstream of the <i>rrnBT</i> in pKDT	This study
pKDT-t <i>Pbgl-bglG</i>	t <i>Pbgl-bglG</i> cloned downstream of the <i>rrnBT</i> in pKDT	This study

References

1. Datsenko, K. A.; Wanner, B. L., One-step inactivation of chromosomal genes in *Escherichia coli* K-12 using PCR products. *Proc Natl Acad Sci U S A* **2000**, 97, (12), 6640-5. DOI: 10.1073/pnas.120163297. PMID: 10829079.
2. Zhang, Z.; Saier, M. H., Jr., A mechanism of transposon-mediated directed mutation. *Mol Microbiol* **2009**, 74, (1), 29-43. DOI: 10.1111/j.1365-2958.2009.06831.x. PMID: 19682247.
3. Zhang, Z.; Zhou, K.; Tran, D.; Saier, M., Insertion Sequence (IS) Element-Mediated Activating Mutations of the Cryptic Aromatic beta-Glucoside Utilization (BglGFB) Operon Are Promoted by the Anti-Terminator Protein (BglG) in *Escherichia coli*. *Int J Mol Sci* **2022**, 23, (3). DOI: 10.3390/ijms23031505. PMID: 35163427.
4. Klumpp, S.; Zhang, Z.; Hwa, T., Growth rate-dependent global effects on gene expression in bacteria. *Cell* **2009**, 139, (7), 1366-75. DOI: 10.1016/j.cell.2009.12.001. PMID: 20064380.

Supplementary Table S2. Oligonucleotides used in this study

Name	Sequence	Use
Pbgl-Xho-F	atactcgagtggcgatgagctggataaactgctg	Cloning <i>Pbgl</i> , <i>Pbgl-bglG</i> or IS5 <i>Pbgl-bglG</i> into pKDT
Pbgl-Bam-R	ttaggatcctgcagtaacaatccagtcatttattaatg	Cloning <i>Pbgl</i> into pKDT
bglG-Bam-R	taggatccttagactatTTTTCTGGCTAACTCCGTC	Cloning <i>Pbgl-bglG</i> , IS5 <i>Pbgl-bglG</i> or t <i>Pbgl-bglG</i> into pKDT
tPbgl-Xho-F	aatctcgagctaaaattacacaaagtaataactg	Cloning t <i>Pbgl-bglG</i> into pKDT
Pbgl-F2	tggcgatgagctggataaactg	Verification of <i>Pbgl</i> cloning
Pbgl-R2	tcagttcatgactgctcaaggcatac	Verification of <i>Pbgl</i> cloning
bglG-ver-F1	agcatggcaatgtgcggaacggatc	Verification of <i>Pbgl-bglG</i> cloning
bglG-ver-F2	ttgtgggtgttattgatgatcaacag	Verification of <i>Pbgl-bglG</i> cloning
bglG-ver-R1	taactgcgtgacacctgcaacatc	Verification of <i>Pbgl-bglG</i> cloning
bglG-ver-R2	tcccaagcgtcctgcgctaaagag	Verification of <i>Pbgl-bglG</i> cloning
IS5R-ver-R1	accttatccgctggaaacctgctac	Verification of IS5 <i>Pbgl-bglG</i> cloning
IS5R-ver-R2	aaggaagaacctgaaacagcatc	Verification of IS5 <i>Pbgl-bglG</i> cloning
Pbgl-Z-P1	gcatttacgttgacaccatcgaatggcgcaaaacctttcgcggtatgtgtaggctgga gctgcttc	Amplification of <i>Pbgl</i> , <i>Pbgl-bglG</i> or IS5 <i>Pbgl-bglG</i> with <i>km^r</i> from pKDT_ <i>Pbgl</i> , pKDT_ <i>Pbgl-bglG</i> or pKDT_ IS5 <i>Pbgl-bglG</i>
Pbgl-Z-P2	cgacggccagtgaaatccgtaatcatggatcatagctgttctctgtgtgaaatgcagtaa caatccagtcatttattaatg	Amplification of <i>Pbgl</i> with <i>km^r</i> from plasmid pKDT_ <i>Pbgl</i>
bglG-Z2	cgacggccagtgaaatccgtaatcatggatcatagctgttctctgtgtgaaattagactat tttctggctaactcc	Amplification of <i>Pbgl-bglG</i> , IS5 <i>Pbgl-bglG</i> or IS5 <i>Pbgl-bglG</i> with <i>km^r</i> from pKDT_ <i>Pbgl-bglG</i> , pKDT_ IS5 <i>Pbgl-bglG</i> or pKDT_ t <i>Pbgl-bglG</i>
hns-AB-F	ctcttcgtgcgcaggcaagagaatgtacacttgaaacgctggaagaaatgctcctaa ttttgttgacactctatc	Amplification and chromosomal integration of <i>tetA:sacB</i>
hns-AB-R	agccgcgctttcttcttcgcgacgttcgtaacaacaacttctaattttcatcaaagg aaaactgtccatgctc	Amplification and chromosomal integration of <i>tetA:sacB</i>
hns-100	cttcgtgcgcaggcaagagaatgtacacttgaaacgctggaagaaat gctg aaaa attagaagttgtgttaacgaacgtcgcgaagaagaaagcgcg	A partial <i>hns</i> fragment carrying the L30P mutation (bolded)
hns-F	cacttaaaattctgaacaacatccgtactcttcgtgcgcaggcaagagaatg	Amplification of hns-100
hns-R	gagtgcgtcttcaacttcagcagcagccgcgctttcttcttcgcgacgttc	Amplification of hns-100
hns-ver-R	agattattgcttgatcaggaaatcgctc	Verification of <i>hns</i> L30P on chromosome