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Measurement of the Promoter Activity in *Escherichia coli* by Using a Luciferase Reporter

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[Abstract] The reporter system is widely used technique for measuring promoter activity in bacterial cells. Until now, a number of reporter system have been developed, but the bioluminescent reporter constructed from the bacterial luciferase genes is one of the useful systems for measuring *in vivo* dynamics of gene expression. The introduced bioluciferase *lux* reporter enables easy, fast, and sensitive measurement of the promoter activity without cell lysis because the substrates of bioluminescent reaction are synthesized inside the bacterial cell, thereby allowing low-cost experiments. This protocol describes a high throughput technique to measure the promoter activity in *Escherichia coli* K-12 using the *lux* reporter system.

Keywords: *lux* operon, Luciferase, *Escherichia coli,* Promoter activity, High-throughput assay, H-NS silencer

[Background] The promoter activity *in vivo* was measured using a reporter system such as the *lacZ* (encoding β-galactosidase), *gus* (encoding β-gulucuronidase), and *cat* (encoding chloramphenicol acetyltransferase) genes. In the case of the *lacZ* reporter system, for instance, the test promoter sequence is fused to a promoter-less *lacZ* gene, creating a test promoter-*lacZ* fusion gene, which is then transferred into a recipient cell. For the measurement of the activity of the test promoter, however, the whole cell lysate must be prepared to detect *in vitro* β-galactosidase activity by adding a substrate such as ONPG (*O*-Nitrophenyl-β-D-galactopyranoside). To avoid such biochemical procedures, the fluorescent *gfp* gene, coding green fluorescent protein (GFP), was employed as a reporter which can be detected without cell lysis. Thus, the fluorescent reporter system is more convenient than the systems which requires measurement of enzymatic activity. However, the fluorescent proteins have a technical limitation especially in genes that are expressed at low levels because of high background noise that arises from intrinsic autofluorescence of cells. To overcome this problem, the luminescent reporter has been developed, which catalyzes bioluminescence reactions using the substrate as luciferins (Meighen, 1991). The *Photorhabdus luminescens* bioluminescence *luxCDABE* genes, coding two luciferase subunits (LuxAB) and three proteins (LuxCDE), which are important for substrate biosynthesis (Bjarnason *et al*., 2003). Once the test promoter is fused to promoter-less *luxCDABE*, both luciferase and its substrate are expressed under the control of the test promoter, and the promoter activity can be easily determined by measuring luminescence without the cell lysis (Bjarnason *et al*., 2003). This

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bioluminescent reporter system is recognized as a powerful high-throughput assay for studying continuous kinetics of promoter activity (Yamanaka *et al.*, 2018; Burton *et al*., 2010). In this protocol, we describe how to construct the bioluminescent reporter system and how to measure the promoter activity in *E. coli*.

Materials and Reagents

1. Pipette tips (Thermo Fisher Scientific, catalog number: QSP Liquid Handling Products 110-Q and 111-Q) (Gilson, catalog number: DIAMOND Tips DL10 and D5000)
2. 1.5 ml plastic tube (Rikaken, catalog number: STAR MicroTestTube 1.5 ml RSV-MTT1.5)
3. 0.22 μm filter (Advantec, catalog number: 13CP020AS)
4. Glass tubes (Iwaki, catalog number: TEST18NP)
5. Sterile 50 ml plastic tube (Iwaki, catalog number: 2345-050)
6. BD Falcon 96-well plates, Black/Clear BD Optilux (Becton Dickinson, catalog number: 353948)
7. 96-well white plate (Becton Dickinson, catalog number: 353377)
8. Petri dishes (Rikaken, catalog number: STAR SDish9015 ver.2 RSU-SD9015-2)
9. pLUX vector (Burton *et al*., 2010)
10. Specific primers (Thermo Fisher Scientific, Custom DNA Oligos) (Table 1)
11. TaKaRa Ex Taq (Takara Bio, catalog number: RR001A)
12. NucleoSpin Gel and PCR Clean-up (Macherey-Nagel, catalog number: 740609.10)
13. Restriction enzyme *Xho* I (Takara Bio, catalog number: 1094)
14. Restriction enzyme *Bam* HI (Takara Bio, catalog number: 1010)
15. In-Fusion HD Cloning Plus (Takara Bio, catalog number: 638920)
16. Kanamycin Monosulfate (Nacalai Tesque, catalog number: 19839-44)
17. Plasmid DNA Extraction Mini Kit (Favorgen, catalog number: FAPDE 001)
18. BigDye® Terminator v3.1 Ready Reaction Mix (Applied Biosystems, catalog number:4337455)
19. IPTG (Nacalai Tesque, catalog number: 06289-67)
20. 3 M sodium acetate (Nacalai Tesque, catalog number: 06893-24)
21. Ethanol (Nacalai Tesque, catalog number: 14710-25)
22. Hi-Di™ Formamide (Applied Biosystems, catalog number: 4311320)
23. Bacto™ tryptone (BD Biosciences, catalog number: 211705)
24. Bacto™ yeast extract (BD Biosciences, catalog number: 212750),
25. NaCl (Nacalai Tesque, catalog number: 31320-05)
26. NaOH (Nacalai Tesque, catalog number: 31511-05)
27. $Na_2HPO_4 \cdot 12H_2O$ (Nacalai Tesque, catalog number: 31722-45)
28. KH_2PO_4 (Wako, catalog number: 498748161612)
29. $MgCl_2 \cdot 6H_2O$ (Nacalai Tesque, catalog number: 20908-65)
30. $K_2SO_4 \cdot 12H_2O$ (Nacalai Tesque, catalog number: 01727-25)
31. NH_4Cl_2 (Nacalai Tesque, catalog number: 02424-55)

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32. $CaCl_2$ (Nacalai Tesque, catalog number: 08894-25)
33. D-(+)-Glucose (Nacalai Tesque, catalog number: 16805-35)
34. 0.5 M EDTA (Nacalai Tesque, catalog number: 06894-14)
35. Competent *E. coli* DH5α, provided from National Institute of Genetics in Japan (preparation at time of use) (see Recipes)
36. 50 mg/ml kanamycin (see Recipes)
37. LB broth (see Recipes)
38. LB agar with 50 μg/ml kanamycin (see Recipes)
39. 125 mM EDTA (see Recipes)
40. 70% ethanol (see Recipes)
41. M9-Glucose medium (see Recipes)

Equipment

1. Pipettes (Gilson, models: PIPETMAN P2, P10, P20, P100, P200, P1000, P5000)
2. Centrifuge (Tomy, model: MX-301)
3. Thermal Cycler (Applied Biosystems, model: 2720Thermal Cycler)
4. Temperature chamber (Taitec, model: Thermo minder SM-10R)
5. Water bath shaker (Taitec, model: Personal-11)
6. DNA sequencer (Applied Biosystems, model: 3500Genetyc Analyzer)
7. Plate reader (Corona, model: MTP−880Lab)
8. Autoclave (Tomy Seiko, model: LSX-500)

Software

1. SF6 for Windows (Corona, in only Japanese)
2. Microsoft Excel (Microsoft)

Procedure

A. Construction of pLUX reporter plasmids
   1. Determine promoter region around a target gene. Typically, we take a region of DNA from 500 base-pairs (bp) upstream to 150 bp downstream of distal transcription start site (see for instance, the determined promoters of 18 target genes Figure 1).

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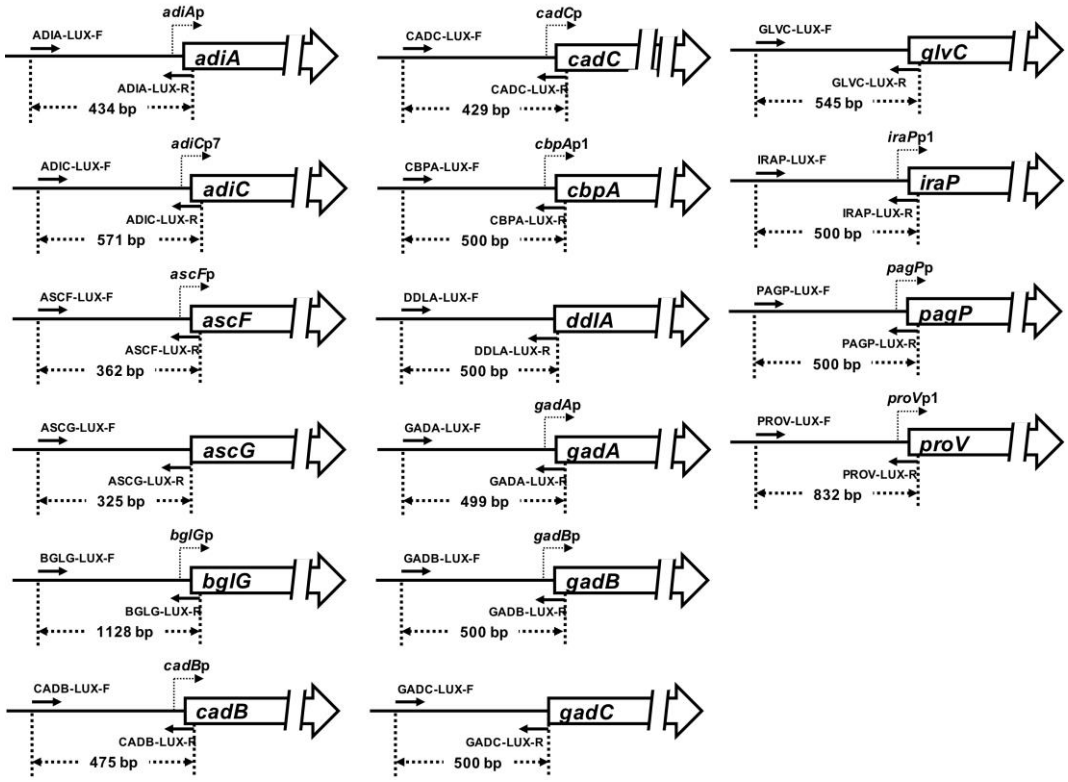

Figure 1. Eighteen *E. coli* promoter regions into pLux

2. Design primers which including 15 overlapping nucleotides (nt) with the pLUX vector for amplification of the determined promoter region (see Figure 2 and Table 1).
3. Amplify target promoter regions by PCR using Ex Taq polymerase (Takara Bio). Standard reaction condition is used as described in the manual for Ex Taq polymerase (Takara Bio). In most of the cases, thermal cycle conditions are as follows: after heating at 98 °C for 5 min, process 30 cycles of 98 °C for 10 sec (denature), 50 °C for 30 sec (annealing), and 72 °C for 1 min (elongation) (Figure 2).
4. Purify the PCR product by NucleoSpin Gel and PCR Clean-up (Macherey-Nagel).
   Note: When PCR product includes non-specific bands, the target product is cut from agarose gel after electrophoresis with total volume of PCR product and then purified by NucleoSpin Gel and PCR Clean-up (Macherey-Nagel).
5. Digest pLUX vector using restriction enzymes *Xho* I and *Bam* HI, and then purify the liner pLUX by NucleoSpin Gel and PCR Clean-up (Macherey-Nagel).
   Note: For cloning into pLUX, we recommend the double-digestion of pLUX by both Xho I and Bam HI (Figure 2).
6. Clone a target promoter as the purified PCR product into the liner pLUX using In-Fusion HD Cloning Plus (Takara Bio).
   Note: We recommend in vivo E. coli cloning (iVEC) as an alternative DNA cloning method (Nozaki et al., 2019), which is provided from National BioResource Project (NBRP) of Japan.
7. Add a part (~10 µl) of the In-Fusion reaction mixture into 0.1 ml of the suspension of competent

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E. coli DH5α (see Recipes), and then incubate the mixture on ice for 30 min.

8. Heat the mixture at 42 °C for 45 sec, and immediately add 0.9 ml of LB broth to the mixture.
9. Incubate 1 ml of the suspension of *E. coli* DH5α transformants at 37 °C for 60 min.
10. Spread 0.1-0.2 ml of the suspension of *E. coli* DH5α transformants onto the LB agar with 50 μg/ml kanamycin, and then incubate the agar plate at 37 °C overnight.
11. Isolate a transformant of *E. coli* DH5α harboring the cloned candidates as a single colony on the LB agar with 50 μg/ml kanamycin.
12. Inoculate a single colony into 5 ml of LB broth containing 0.05 ml of 50 mg/ml kanamycin in a glass tube (Iwaki), and incubate the culture in water bath (Taitec) at 37 °C with shaking (120 rpm) overnight.
13. Isolate the plasmid from the transformant cells of 5 ml overnight culture by Plasmid DNA Extraction Mini Kit (Favorgen).
14. Sanger reaction is performed with the isolated plasmid as a template, LUX-R primer (Table 1), and BigDye® Terminator v3.1 Ready Reaction Mix (Applied Biosystems) according to the recommended procedure by Supplier.
15. Transfer 20 μl of sanger product into a sterile 1.5 ml plastic tube and then add 2 μl of 125 mM EDTA, 2 μl of 3 M sodium acetate, and 50 μl of ethanol (Nacalai Tesque).
16. Mix the solution by vortex mixer for 15 sec.
17. Collect DNA pellet by centrifugation (17,800 *x g*, 4 °C, 15 min).
18. Add 70 μl of 70% ethanol and collect DNA pellet by centrifugation (17,800 *x g*, 4 °C, 15 min).
19. Dissolve DNA pellet by 15 μl of Hi-Di™ Formamide (Applied Biosystems).
20. Determine DNA sequence of the promoter region cloned into the isolated plasmid with DNA sequencer (Applied Biosystems).

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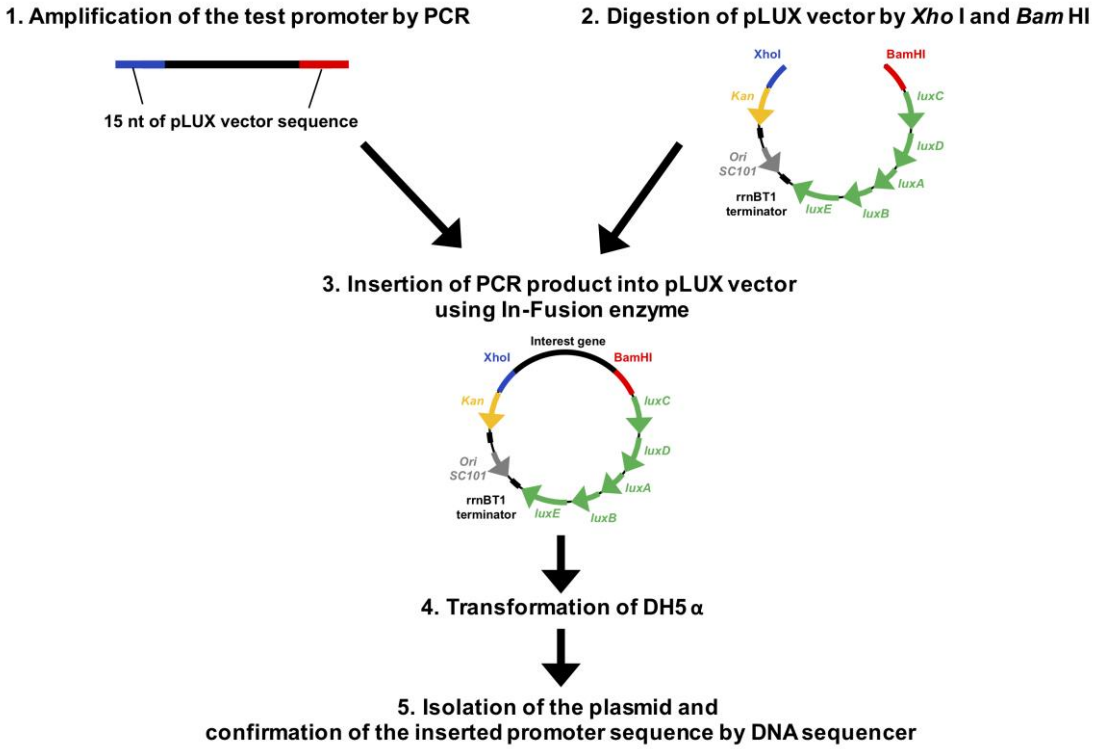

Figure 2. Strategy for construction of pLUX reporter plasmid. The promoter region was amplified by PCR using the *E. coli* K-12 genome as the template and a pair of specific primers (Table 1). The amplified DNA fragment was inserted into a pLUX vector using the In-Fusion HD cloning kit (Takara Bio). After transformation of DH5α, the plasmid was purified from a culture of transformant cells. The DNA sequence of insertion on the resulting plasmids was confirmed by DNA sequencing.

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Table 1. The used primers and pLUX derivatives

Name	Characterization	Reference
Primers		
ADIA-LUX-F	5'- TCGTCTTCACCTCGAATGGGGATATTCCAGCGGGTCATGC -3' (for amplification of *adiA* promoter)	This study
ADIA-LUX-R	5'- ACTAACTAGAGGATCCATTGCTTACCCGGTTATGAAGGAA -3' (for amplification of *adiA* promoter)	This study
ADIC-LUX-F	5'- TCGTCTTCACCTCGAGAAACTGAGTCAGAAAAGGAACGAA -3' (for amplification of *adiC* promoter)	This study
ADIC-LUX-R	5'- ACTAACTAGAGGATCAATTAAACTCCTGCGAAGGCGAGCT -3' (for amplification of *adiC* promoter)	This study
ASCF-LUX-F	5'- TCGTCTTCACCTCGAGCACGCGGGAAACGGTCGCTTTTGA -3' (for amplification of *ascF* promoter)	This study
ASCF-LUX-R	5'- ACTAACTAGAGGATCCTATCACCGAGCGTGCCAGCGCCGC -3' (for amplification of *ascF* promoter)	This study
ASCG-LUX-F	5'- TCGTCTTCACCTCGAGATGTTATCAACGCCGCCCAGTGCC -3' (for amplification of *ascG* promoter)	This study
ASCG-LUX-R	5'- ACTAACTAGAGGATCCCCCCGGCGCGCTTCGCCACTTCCAG -3' (for amplification of *ascG* promoter)	This study
BGLG-LUX-F	5'- TCGTCTTCACCTCGAGACAAATAATTCACCAGACA -3' (for amplification of *bglG* promoter)	This study
BGLG-LUX-R	5'- ACTAACTAGAGGATCGTGTTCTTTGCGCACGCGCT -3' (for amplification of *bglG* promoter)	This study
CADB-LUX-F	5'- TCGTCTTCACCTCGATTTAATTTACGCCCAGGGGCAAACA -3' (for amplification of *cadB* promoter)	This study
CADB-LUX-R	5'- ACTAACTAGAGGATCGCTCTTCTCCTAATTTCATTTTTGA -3' (for amplification of *cadB* promoter)	This study
CADC-LUX-F	5'- TCGTCTTCACCTCGACGTGCGGCCCCGTGATGCTGTTGAA -3' (for amplification of *cadC* promoter)	This study
CADC-LUX-R	5'- ACTAACTAGAGGATCAATAGAAACTCATTCGAAAAGGGAA -3' (for amplification of *cadC* promoter)	This study
CBPA-LUX-F	5'- TCGTCTTCACCTCGAATTATCATTCTGCATTTCCTCAAAT -3' (for amplification of *cbpA* promoter)	This study
CBPA-LUX-R	5'- ACTAACTAGAGGATCAGCGTTATCTCGCGTAAATCAACAC -3' (for amplification of *cbpA* promoter)	This study
DDLA-LUX-F	5'- TCGTCTTCACCTCGAGCAAGCTTAAATAACAACTCAGCAA -3' (for amplification of *ddlA* promoter)	This study
DDLA-LUX-R	5'- ACTAACTAGAGGATCCTTAAAAACCTATCCCGTCTAACAC -3' (for amplification of *ddlA* promoter)	This study
GADA-LUX-F	5'- TCGTCTTCACCTCGAGAAAAAAGACTTTAACTTTGGGGAA -3' (for amplification of *gadA* promoter)	This study
GADA-LUX-R	5'- ACTAACTAGAGGATCTTCGAACTCCTTAAATTTATTTGAA -3' (for amplification of *gadA* promoter)	This study
GADB-LUX-F	5'- TCGTCTTCACCTCGATCAATATGACGATCCTGCAGC -3' (for amplification of *gadB* promoter)	This study
GADB-LUX-R	5'- ACTAACTAGAGGATCTTTAAACTCCTTAAAATGAT -3' (for amplification of *gadB* promoter)	This study
GADC-LUX-F	5'- TCGTCTTCACCTCGACTGGCGGATGAAATCGCCAAACTGG -3' (for amplification of *gadC* promoter)	This study
GADC-LUX-R	5'- ACTAACTAGAGGATCATTATCCCCCTAAAACGGTATTCCT -3' (for amplification of *gadC* promoter)	This study
GLVC-LUX-F	5'- TCGTCTTCACCTCGAGCGCACCAGGTAAGTGCCACTACCG -3' (for amplification of *glvC* promoter)	This study
GLVC-LUX-R	5'- ACTAACTAGAGGATCGCACTGGCGTGAACATCGCGCCGCC -3' (for amplification of *glvC* promoter)	This study
IRAP-LUX-F	5'- TCGTCTTCACCTCGATTACCACCAAAAACGATTCCTACCC -3' (for amplification of *iraP* promoter)	This study
IRAP-LUX-R	5'- ACTAACTAGAGGATCGTCTGTATTTCCTTATCCAAAGTAT -3' (for amplification of *iraP* promoter)	This study
PAGP-LUX-F	5'- TCGTCTTCACCTCGAGCTGATTAAAATCAAGAAAAACTGC -3' (for amplification of *pagP* promoter)	This study
PAGP-LUX-R	5'- ACTAACTAGAGGATCTTGTGACCATAAAACATTTATCAAA -3' (for amplification of *pagP* promoter)	This study
PROV-LUX-F	5'- TCGTCTTCACCTCGAATCTCTGGGACAACGTGAAG -3' (for amplification of *proV* promoter)	This study
PROV-LUX-R	5'- ACTAACTAGAGGATCCCAGACTGGCGTCTTTTACG -3' (for amplification of *proV* promoter)	This study
Lux-R	5'-GGCAGGTAAACACTATTATCACC-3'	Yamanaka *et al*., 2014
pLUX derivatives		
pLUX	promoter-less *luxCDABE*	Burton *et al*., 2010
pLUXgadWp	pLUX, *gadW'-lux*	Burton *et al*., 2010

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pLUXslpp	pLUX, *slp'-lux*	Burton *et al*., 2010
pLUXadiAp	pLUX, *adiA-lux* (the *adiA* promotert [434 bp] is -399 to +35 from transcription start site of *adiA*p)	This study
pLUXadiCp	pLUX, *adiC-lux* (the *adiC* promotert [571 bp] is -400 to +171 from transcription start site of *adiC*p7)	This study
pLUXascFp	pLUX, *ascF-lux* (the *ascF* promotert [362 bp] is -228 to +134 from transcription start site of *ascF*p)	This study
pLUXascGp	pLUX, *ascG-lux* (the *ascG* promotert [325 bp] is -325 to -1 from transcription start site of *ascG*p)	This study
pLUXbglGp	pLUX, *bglG-lux* (the *bglG* promotert [1128 bp] is -164 to +964 from transcription start site of *bglG*p)	This study
pLUXcadBp	pLUX, *cadB-lux* (the *cadB* promotert [475 bp] is -400 to +75 from transcription start site of *cadB*p)	This study
pLUXcadCp	pLUX, *cadC-lux* (the *cadC* promotert [429 bp] is -400 to +29 from transcription start site of *cadC*p)	This study
pLUXcbpAp	pLUX, *cbpA-lux* (the *cbpA* promotert [500 bp] is -442 to +58 from transcription start site of *cbpA*p1)	This study
pLUXddlAp	pLUX, *ddlA-lux* (the *ddlA* promotert [500 bp] is -500 to -1 from the fist nucleotide of translation start codon of *ddlA*)	This study
pLUXgadAp	pLUX, *gadA-lux* (the *gadA* promotert [499 bp] is -472 to +27 from transcription start site of *gadA*p)	This study
pLUXgadBp	pLUX, *gadB-lux* (the *gadB* promotert [500 bp] is -473 to +27 from transcription start site of *gadB*p)	This study
pLUXgadCp	pLUX, *gadC-lux* (the *gadC* promotert [500 bp] is -500 to -1 from the fist nucleotide of translation start codon of *gadC*)	This study
pLUXglvCp	pLUX, *glvC-lux* (the *glvC* promotert [545 bp] is -496 to +49 from the fist nucleotide of translation start codon of *glvC*)	This study
pLUXiraPp	pLUX, *iraP-lux* (the *iraP* promotert [500 bp] is -433 to +67 from transcription start site of *iraP*p1)	This study
pLUXpagPp	pLUX, *pagP-lux* (the *pagP* promotert [500 bp] is -469 to +31 from transcription start site of *pagP*p)	This study
pLUXproVp	pLUX, *proV-lux* (the *proV* promotert [832 bp] is -432 to +400 from transcription start site of *proV*p1)	This study

The plasmids constructed in this study could be provided from National BioResource Project (NBRP) *E. coli* of Japan.

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B. Measurement of luciferase activity in *E. coli*

1. Transform *E. coli* strains by the cloned luciferase reporter plasmids.

   Note: The ΔhnsΔhhaΔydgT strain, isolated from E. coli K-12 W3110 strain, deleted three genes, hns, hha, and ydgT, in its genome (Ueda et al., 2013). H-NS plays a role in transcriptional silencing of genes, which is modulated by Hha and YdgT proteins in E. coli. The ΔhnsΔhhaΔydgT strain was transformed with pQE80L, pQE80Lhns which carries hns gene in pQE80L, pQE80Lhns-I70A which carries hns gene with substitutions of Ile70Ala, or pQE80Lhns-L75A which carries hns gene with substitutions of Leu75Ala. These transformants were used as hosts for luciferase measurements.

2. Inoculate at least three single colonies of *E. coli* transformant in glass tubes separately under M9-glucose medium including 50 μg/ml kanamycin.

   Note: In addition of kanamycin, ampicillin should be added in medium at the final concentration of 100 μg/ml for the ΔhnsΔhhaΔydgT strain harboring a pQE80L derivative and a pLux derivative.

3. Incubate the pre-cultures at 37 °C with shaking for overnight.
4. Inoculate 100 μl of overnight pre-culture in 10 ml of fresh M9-glucose medium including 50 μg/ml kanamycin.
5. Incubate the 10 ml cultures at 37 °C in water bath (Taitec) with shaking (120 rpm) until luciferase activity is measured.
6. Transfer 100 μl of culture to a well of a Black/Clear BD Falcon 96-well plate (Becton Dickinson) in triplicate for each culture.

   Note: We tested 96-well plates for measuring luminescence in E. coli with the plate reader (Corona), indicating that the 96-well white plate showed the leakage of 1.5% to an adjacent empty well whereas the 96-well black plate reduced leakage by 0.95% (see the row data in both 96 plates in Figure 3). Although a white plate is usually used for luminescent measurements, a black plate was used to prevent leakage of high luminescence from one culture to other well in this study. Additionally, during measurement we arranged cultures with a single well gap between them to minimize leakage to adjacent wells. However, we recommend that a white plate be used first as standard procedure.

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1. The measured intensity of luciferase activity in *E. coli* in 96 well black plate (Becton Dickinson, catalog number: 353948)

	1	2	3	4	5	6	7	8	9	10	11	12
A	136,932	178,982	140,691	517								
B	924	959	792	−239								
C												
D												
E												
F												
G												
H					−375	−268	−220		−383	−77	−205	

2. The measured intensity of luciferase activity in *E. coli* in 96 well white plate (Becton Dickinson, catalog number: 353377)

	1	2	3	4	5	6	7	8	9	10	11	12
A	363,077	473,103	359,535	23,512								
B	19,944	20,826	19,223	14,633								
C												
D												
E												
F												
G												
H					14,720	14,231	14,500		13,816	13,617	13,094	

Figure 3. The measured intensity of luciferase activity in *E. coli* in 96 well plates. *E. coli* K-12 W3110 (parent strain) harbouring pLUXappYp, containing *appY-lux* operon (data not shown) was grown in M9-glucose medium at 37 °C, and culture was applied into three wells (A1, A2, and A3) of both the 96-well black plate (Becton Dickinson, catalog number: 353948) (upper) and the 96-well white plate (Becton Dickinson, catalog number: 353377) (lower). M9-glucose medium (H5, H6, and H7) and distilled water (H9, H10, and H11) were also applied into three wells of both the 96 well plates. Each intensity is shown as a raw data measured with the plate reader (Corona). The fluorescent intensity was detected in the empty wells adjacent to wells filled by culture (A4 and B1 to B4).

7. Transfer 100 µl of fresh M9-glucose medium to another well of the Black/Clear BD Falcon 96 well plate (Becton Dickinson) used in Step B6 in triplicate for background.
8. Set the Black/Clear BD Falcon 96 well plate (Becton Dickinson) containing the samples and the background with plate reader (Corona).
9. Measure OD_{600} according to the procedure for the plate reader (Corona).
10. Measure luminescence as a total intensity. Therefore, no filter is set according to the procedure for the plate reader (Corona).

Data analysis

1. Extract raw numeric data of both the values of OD_{600} and the intensities of luminescence as a text file from SF6 for Windows (Corona).
2. Open a text file containing the raw numeric data in Microsoft Excel (Microsoft).
3. Normalize the net values of OD_{600} and the net intensities of luminescence with background.
4. Calculate the ratio of luminescence to OD_{600} as specific activity of the promoter of each culture by the following formula: the net intensity of luminescence/the net values of OD_{600}.

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5. Average the ratios of triplicate with standard deviation.

   Note: The activities of the 18 promoters used are represented in Figure 4, indicating that all 18 promoters silenced by H-NS were de-silenced by two H-NS mutants in agreement with our previous work (Yamanaka et al., 2018). To confirm a significant difference, calculated p-values by t-test of statistical analysis should be evaluated at less than 0.01.

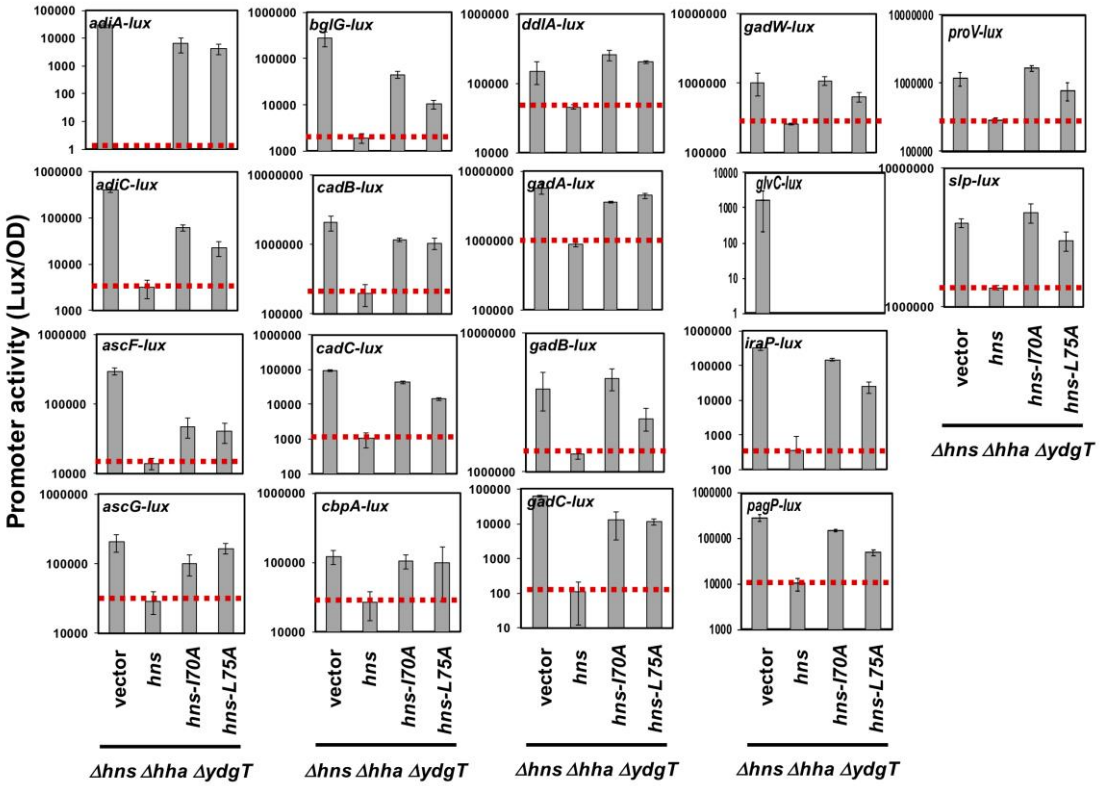

Figure 4. Luciferase reporter reveals silence of 18 promoters by H-NS in *E. coli*. The constructed 18 pLux reporter plasmids, pLUXadiAp, pLUXadiCp, pLUXascFp, pLUXascGp, pLUXbglGp, pLUXcadBp, pLUXcadCp, pLUXcbpAp, pLUXddlAp, pLUXgadAp, pLUXgadBp, pLUXgadCp, pLUXgadWp, pLUXglcVp, pLUXiraPp, pLUXpagPp, pLUXproVp, and pLUXslpp (Table 1), were introduced into *ΔhnsΔhhaΔydgT* strains harbouring the *hns* plasmids (pQE80Lhns, pQE80Lhns-I70A, and pQE80Lhns-L75A) or an empty plasmid (a vector pQE80L). Transformants were grown in M9-glucose medium with 10 μM IPTG at 37 °C, and then the promoter activity was calculated as described above.

Recipes

1. Competent *E. coli* DH5α
   a. Inoculate a single colony of *E. coli* DH5α in 5 ml of LB broth
   b. Incubate the pre-cultures at 37 °C with shaking for overnight
   c. Dilute overnight pre-culture 100-fold in 10 ml of fresh LB broth

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d. Incubate the cultures at 37 °C with shaking until mid-logarithmic phase
e. Transfer 10 ml of culture to a sterile 50 ml plastic tube (Iwaki)
f. Collect the cells by centrifugation (2,300 *x g*, 4 °C, 10 min)
g. Suspend the cells in 10 ml of cold 0.1 M $CaCl_2$
h. Incubate the cells on ice for 30 min.
i. Collect the cells by centrifugation (2,300 *x g*, 4 °C, 10 min)
j. Suspend the cells in 1 ml of cold 0.1 M $CaCl_2$
k. Use a part (~100 µl) of the resuspended *E. coli* DH5α for transformation

2. 50 mg/ml kanamycin
   a. Dissolve 0.5 g of kanamycin monosulfate (Nacalai Tesque) in 10 ml of distilled water
   b. Sterilize the solution by filtration with 0.22 µm filter (Advantec)
   c. Store the sterilized solution at 4 °C
3. LB broth
   a. Dissolve 10 g of Bacto™ tryptone, 5 g of Bacto™ yeast extract, and 10 g of NaCl in 800 ml of distilled water
   b. Adjust pH to 7.5 with NaOH
   c. Adjust volume to 1 L with distilled water
   d. Autoclave the solution (set 121 °C and 20 min in LSX-500)
   e. Store the autoclaved LB broth at room temperature
4. LB agar with 50 µg/ml kanamycin
   a. Dissolve 7.5 g of agar in 1 L of LB broth
   b. Autoclave (set 121°C and 20 min in LSX-500)
   c. Add kanamycin after cooling in the final concentration of 50 µg/ml
   d. Pour the media into Petri dishes, and then harden LB agar by cooling at room temperature
   e. Store the LB agar at 4 °C
5. 125 mM EDTA
   a. Dilute 0.5 M EDTA (Nacalai Tesque) to 125 mM with a sterile water
   b. Store at room temperature
6. 70% ethanol
   a. Dilute ethanol (Nacalai Tesque) to 70% with a sterile water
   b. Store at room temperature
7. M9-Glucose medium (to autoclave, set 121°C and 20 min in LSX-500)
   a. For 5x M9 salt (-NH_4Cl_2), dissolve 75 g of $Na_2HPO_4{\cdot}12H_2O$, 15 g of KH_2PO_4 and 0.25 g of NaCl in 1 L distilled water, and then the dissolved solution was autoclaved
   b. Prepare the autoclaved following solutions: 2 M NH_4Cl_2, 1 M $MgCl_2$, 0.25 M K_2SO_4, 10 mM $CaCl_2$
   c. Sterilize the 1 M glucose by filtration (0.22 µm filter)
   d. Add 200 ml of 5x M9 salt (-NH_4Cl_2), 10 ml of 2 M NH_4Cl_2, 1 ml of 1 M $MgCl_2$, 1 ml of 0.25 M K_2SO_4, 10 ml of 10 mM $CaCl_2$, 10 ml of 1 M Glucose into 768 ml of autoclaved distilled

water

e. Store the sterilized M9-glucouse medium at room temperature

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Competing interests

The authors declare no conflicts of interest.

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