SUPPLEMENTAL MATERIAL

Histone-like nucleoid-structuring protein (H-NS) paralogue StpA activates the type I-E CRISPR-Cas system against natural transformation in *Escherichia coli*

Running title: StpA regulates the type I-E CRISPR-Cas system

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SUPPLEMENTAL MATERIALS AND METHODS

1. Construction of *E. coli* mutants

E. coli mutants were constructed by using the λ-RED recombination system that had been constructed in the temperature sensitive plasmid pKD46 (1). To induce the λ-RED system, arabinose (30 mM) was added to the *E. coli* pKD46 culture at an OD_{600} of 0.2. Chemical competent cells were prepared when the cell culture was grown to an OD_{600} of 0.4. The DNA template containing an antibiotic resistant gene (Cm^R or Kan^R) from either pKD3 or pKD4 for double crossover was PCR amplified with a pair of primers (P_{01}-P_{02} for inactivating *stpA* or P_{03}-P_{04} for inactivating *hns*) containing short homologous arms. The *stpA* and *hns* null mutants were checked by using colony PCR with primer pairs P_{05}-P_{06} for *ΔstpA* and P_{07}-P_{08} for *Δhns*. The temperature sensitive plasmid pCP20 was transformed into the mutant for eliminating the antibiotic resistance gene in the genome. All primers are shown in Table 2.

2. Plasmid construction

The plasmid pCR1 was constructed by inserting the chemically synthesized DNA fragment (CR1) with four protosapcer adjacent motif (PAM)-containing DNA regions, that can be recognized by the CRISPR-Cas system of *E. coli*, into the vector pUC57 at the *Hind* III and *EcoR* I sites. DNA synthesis and cloning were performed by GENEWIZ Biotech Co., LTD. The complete sequence of pCR1 was provided in the section of DNA SEQUENCE INFORMATION at the end of the file.

To construct pGLO-P_{cas}-*gfp* for monitoring transcription of *cas* operon, the promoter of the *cas* operon was fused with the green fluorescence protein (GFP) encoding gene. The fragment of P_{cas} was amplified from *E.coli* genome with primer pairs P_{11} and P_{12}. The linearized vector, that was obtained by PCR amplification of pGLO with primer pairs P_{09} and P_{10}, was recombined with the above fragments by using the One Step Cloning Kit (Qingke Biotech Co., LTD).

To construct pGLO-P_{rscA}-*gfp* for checking transcriptional activity of a promoter known to be regulated by H-NS and StpA, the fragment of P_{rscA} was amplified from *E.coli* genome with primer pairs P_{15} and P_{16}. The linearized vector, that was obtained through PCR amplification of pGLO with primer pairs P_{13} and P_{14}, was recombined with the above fragments by using the One Step Cloning Kit (Qingke Biotech Co., LTD).

The plasmid pGLO-P_{cas}^{*}-*gfp* carrying mutations in the putative DNA binding site (DBS) for H-NS/StpA was constructed to identify the DBS for H-NS/StpA regulation in P_{cas}. Mutations in DBS was achieved by using site-directed mutagenesis with pGLO-P_{cas}-*gfp* as the template and the primer pair P_{25}-P_{26} for amplification.

To construct pSUStpA and pSUHNS for ectopic expressing StpA and H-NS on plasmid, DNA fragments containing *stpA* and *hns* together with their original promoters were amplified with primer pairs P_{17}-P_{18} and P_{21}-P_{22} from the *E.coli* genome. The linearized vectors, that was obtained by PCR amplification of pSU19 with primer pairs P_{19}-P_{20} and P_{23}-P_{24}, were recombined with the above fragments by using the One Step Cloning Kit (Qingke Biotech Co., LTD).

To construct pSU-P_{BAD}-*gfp* which expressed the green fluorescence gene with an arabinose-inducible promoter P_{BAD}, the fragment containing *gfp* together with P_{BAD} was amplified with primer pairs P_{30}-P_{31} from pGLO-*gfp*. The linearized vector, that was obtained by PCR amplification of pSU19 with primer pairs P_{32}-P_{33}, was recombined with the above fragment by using the One Step Cloning Kit (Qingke Biotech Co., LTD). To construct pSU-P_{BAD}-*stpA* and pSU-P_{BAD}-*hns*, fragments containing *stpA* and *hns* were amplified from *E.coli* genome with primer pairs P_{36}-P_{37} and P_{38}-P_{39}. The linearized vector, that was obtained by PCR amplification of pSU19 with primer pairs P_{35}-P_{36}, was recombined with the above fragments by using the One Step Cloning Kit (Qingke Biotech Co., LTD).

3. Quantification of crRNA with RT-qPCR

Overnight-grown *E. coli* culture (1 ml) was precipitated and the cell pellet was resuspended in 1 ml RNA trizol (TransGen Biotech Co., LTD). The cell resuspension was mixed thoroughly with 0.2 ml chloroform. After incubation at the room temperature for 3 minutes, the mixture was centrifugated at 10, 000 rpm for 15 minutes. The upper layer of the mixture was transferred to the RNA spin column for purifying the total RNA, which was retained in the eluate. Total RNA in the eluate was transferred to a miRNA spin column, in which the resin matrix had high affinity for small RNA (sRNA). The attached sRNA on the resin matrix was washed with ethanol and the washing buffer provided in the EasyPure® miRNA Kit (TransGen Biotech Co., LTD). Then, 30 μL RNase-free water was added to the miRNA spin column to dissolve sRNA which was subsequently examined by gel electrophoresis before being stored at -80℃.

For quantifying crRNA, the first strand of the corresponding cDNA was obtained with the Trans*Script*® miRNA First-Strand cDNA Synthesis SuperMix Kit (TransGen Biotech Co., LTD). The reaction system (10 μl) for reverse transcription was as follows: sRNA (0.2 mg ml^{-1}) 4.5 μl, *TransScript* miRNA RT Enzyme Mix 0.5 μl, 2 × TS miRNA Reaction Mix 5 μl. The reaction system was left at 37℃ for an hour before inactivation of the RT Enzyme Mix at 85℃ for 5 seconds. During reverse transcription, the DNA fragment (GATCGCCCTTCTACGTCGTATCGTCATCTGACCGTTATCGCTGCAC GTTTTTTTTTTTTTTTTTTT) was simultaneously added to the 3' terminus of the cDNA.

To quantify sRNA transcripts, real-time PCR was performed with a pair of primers (P_{28} and P_{29} for crRNA, P_{27} and P_{29} for tRNA) that were complementary to the target cDNA and the added 3' tail respectively, by using the program as follows: 94℃, 30 seconds; 94℃, 5 seconds; 57℃, 15 seconds; 72℃, 10 seconds; 72.5℃, 5 seconds for melt curve evaluation; recycle number for 40 times. PCR products were examined by using 2% agar gel electrophoresis.

4. Drop-plating

Drop plating was performed according to a documented method (2). The overnight grown culture of *E. coli* carrying pCR1 or pDsRED was serially diluted with LB medium. An aliquot of 10 μl of the diluted cell suspension was dispensed into LB-agar plate supplemented with 100 μg ml^{-1} ampicillin. After drops on the agar dried, the petri plates were inverted and incubated at 30 ℃ for 24-48 hours.

5. Plasmid loss assay in transformants

After natural transformation of *E. coli*, transformant colonies were further grown in LB broth or on LB-agar plates. Cell growth in LB broth was measured in a Spectrumlab S23A Spectrophotometer at 600 nm. To evaluate plasmid loss in transformants, bacteria grown in LB broth were streaked on plates or serial diluted before drop plating (2). To quantify the rate of plasmid loss, numbers of viable counts on LB-agar plates with and without ampicillin (100 μg ml^{-1}) were measured by using the track-dilution cfu enumeration technique (3).

6. Chemical Transformation

Overnight grown *E.coli* cell culture (1 ml) was inoculated into 50 ml LB medium and incubated at 30°C with shaking to an optical density at 600 nm (OD_{600}) of 0.4. The cell pellet, which had been collected by centrifugation and washed twice with 10 ml 100 mM $CaCl_2$ solution on ice, was resuspended in 500 μl of 100 mM $CaCl_2$ solution supplemented with 10% (v v^{-1}) glycerol and restored at -80°C. Plasmid pDsRED or pCR1 was added to 50 μl of the competent cell suspension to a final concentration of ~ 10 μg ml^{-1}, which was placed on ice for 30 minutes before a heat shock at 42°C for 90 seconds. Then, the cell suspension was placed on ice for ~ 1 minute, followed by the addition of 0.5 mL LB. Transformed cells in LB medium were further incubated at 30°C for 2 hours before being spread on LB-agar plates containing ampicillin at a

concentration of 100 μg ml^{-1}. The number of transformants on plates were counted and transformation efficiency was calculated as the number of transformants per μg plasmid.

7. Protein isolation and purification

E.coli BL21 containing pET28a-StpA-His/pET28a-HNS-His was incubated in 50 ml LB medium at 37 ℃ with shaking. To induce the expression of StpA-His/HNS-His, lactose (0.4%) was added to the cell culture at an OD_{600} of 0.4 and further incubated for 6 hours. *E. coli* mutants (*ΔstpA*, *Δhns* and *Δhns ΔstpA*) and their wildtype parent were grown to the stationary phase in LB broth. Cell pellets were collected by centrifugation and washed with the phosphate-buffered saline (PBS) buffer before being resuspended in 5 mL cold PBS buffer. To obtain total proteins, the cell resuspension was sonicated on ice for 20 min (9 s sonication, 9 s interval) at 33% amplitude. StpA-His/HNS-His was purified from total proteins with Tagged Protein Purification Kit (Soluble Protein) Kit (Beijing ComWin Biotech Co., Ltd).

8. Western blot assay

For determination of expression level of StpA/H-NS in cells, total proteins were taken from cultures that had been grown to the stationary phase (10-hour in LB medium and 24-hour in M9 medium), and resuspended in the PBS buffer. Samples were subjected to SDS-PAGE (12% polyacrylamide) before being blotted onto a 0.1 μm nitrocellulose transfer membrane (Bio-rad). The chemically synthesized peptide CAAPRAGKKRQPR (Hangzhou HuaAn Biotechnology Co., Ltd.) was used to raise

the StpA antibody in rabbits which were reviewed and approved by the Animal Research Committee at Zhejiang University of Technology. The polyclonal StpA antibody was used as the primary antibody. HRP-conjugated Goat anti-Rabbit IgG was used as the secondary antibody at a dilution of 1:2000. The treatment of the membrane was as described in Sambrook and Russell (4).

9. Quantification of transcription of *cas* genes with GFP

Cell growth was monitored spectrophotometrically at an optical density of 600 nm (OD_{600}). Intensity of the culture fluorescence, as an indicator of transcription of P_{cas}, was measured by the SpectraMax Gemini EM microplate reader with excitation and emission wavelengths at 395 and 509 nm respectively. Relative gene expression was calculated through dividing the intensity of the culture fluorescence by OD_{600}.

10. Natural transformation of *E. coli hns stpA* carrying pSU-P_{BAD}-*stpA*

All experiments were performed at 30 ℃. The overnight-grown culture of *E. coli hns stpA* mutant carrying pSU-P_{BAD}-*stpA* was added to 50 ml of 1.5 × LB medium with a ratio of 1: 50 (v v^{-1}). After 24-hour incubation with shaking at a speed of 180 rpm, 2 ml of the cell culture was added to each glass tube which was supplemented without or with arabinose at concentrations of 0.5, 1 or 2 mM, followed by further incubation with shaking for 1 hour. After centrifugation at room temperature, 90% of the supernatant was discarded and the cell pellet was resuspended in the remaining 10% supernatant before adding the plasmid pCR1 to a final concentration of 40 μg ml^{-1}. The mixture of

the cell culture and pCR1 was spread on 5% agar (Bacto Difco)- and ampicillin-containing (100 μg ml^{-1}) plates as previously documented (5, 6), without or with arabinose at concentrations of 0.5, 1 or 2 mM. Plates were incubated for two days before counting the number of transformants. Transformation efficiency was calculated as the number of transformants per microgram plasmid DNA.

11. Quantification of cellular StpA:DNA ratio for transcriptional activation.

The *in vivo* cellular StpA : DNA ratio for activating P_{cas} was estimated from the cell culture grown in M9 medium supplemented with 1 mM arabinose. The concentration of the purified StpA-His was ~ 0.25 mg ml^{-1}, determined by the BCA protein assay kit (Beijing ComWin Biotech Co., Ltd). According to the Western Blot assay (FIG 5F), the concentration of StpA isolated from the cell culture grown in M9 medium was about 10-fold lower than that of the purified StpA-His. Providing that the total protein from 50 ml of the cell culture was resuspended in 5 ml of PBS buffer, the amount of StpA in the total protein was estimated to be 0.025 mg ml^{-1} × 5 ml. The viable cell density assayed by plate counting was ~ 5.4×10^9 CFU ml^{-1} in M9 medium. Accordingly, cellular concentration of StpA was about 4.6×10^{-16} g per cell. Therefore, each cell contained 1.8×10^4 StpA molecules. Providing the size of the *E. coli* genome is 4.2×10^6 bp, the StpA:DNA ratio was estimated to be ~ 1: 200.

SUPPLEMENTAL RESULTS & DISCUSSION

1. PCR detection and western blot assay of *E.coli* mutants

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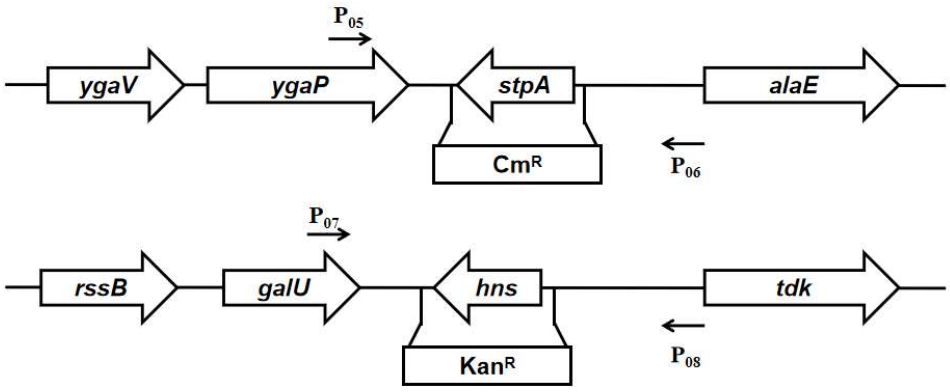

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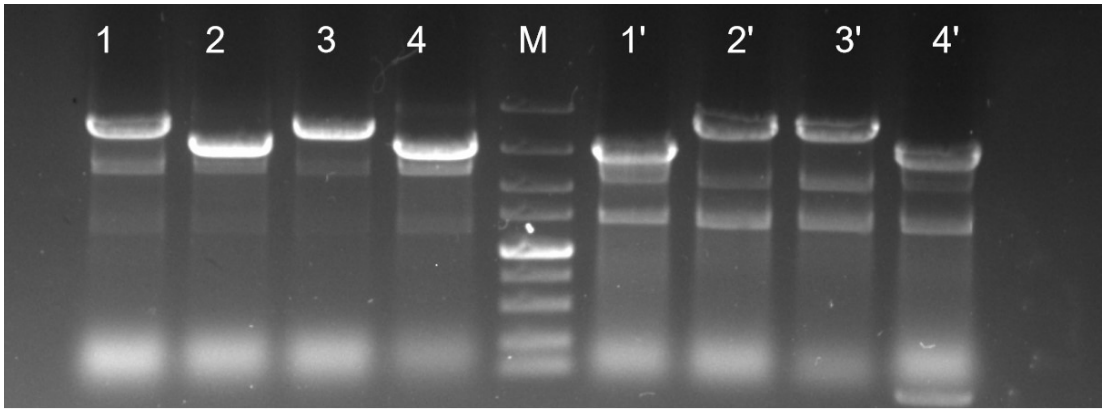

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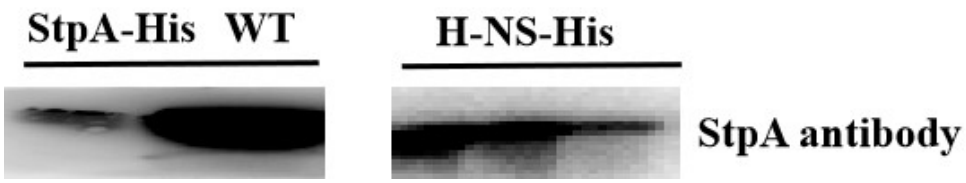


D

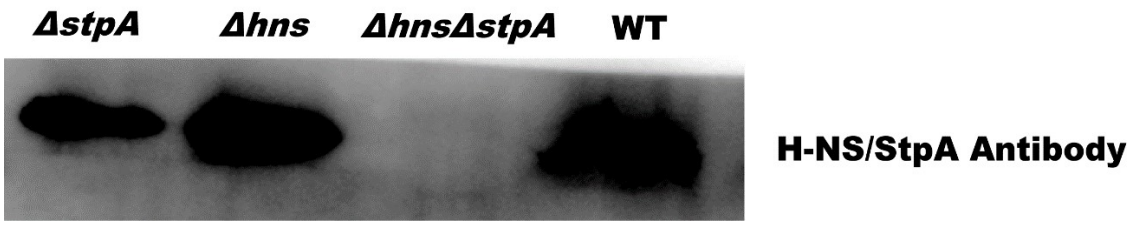


FIG S1. Examination of *E. coli* mutants in this work. (A) Genetic organization of the various chromosomal regions and location of cassette insertion mutants generated in this study (see accompanying TABLE S1). (B) Analysis of PCR fragments for confirmation of the structure of *E. coli* mutant strains. From left to right, each pair of channels compares wild type and mutant strains, as follows. Channel 1/1': *ΔstpA* mutant; Channel 2/2': *Δhns* mutant; Channel 3/3': *Δhns ΔstpA* mutant; Channel 4/4': wild type; M: 5000 DL marker (from the top down: 5 kb, 3 kb, 2 kb, 1.5 kb, 1 kb, 750 bp, 500 bp, 250 bp, 100 bp). (C) Western blot assay was performed to determine interaction between the antibody, raised by a peptide derived from StpA, and purified StpA and H-NS. StpA-His: purified His-tagged StpA protein; H-NS-His: purified His-tagged H-NS protein; WT: Total protein isolated from the wildtype *E. coli* MC4100. (D) Total proteins were isolated from mutants (*ΔstpA*, *Δhns* and *Δhns ΔstpA*) and their wildtype parent grown to the stationary phase in LB broth. Western blot assay was performed with the antibody against H-NS or StpA. In *stpA* and *hns* single-deletion mutants, clear bands were detected, showing the presence of H-NS or StpA in these mutants. Whereas, no band was detected in the *hns stpA* null mutant, revealing that neither H-NS nor StpA was present in that mutant.

TABLE S1 Prediction of the structure of *E.coli* mutant strains generated in this work (see accompanying FIG S1C)

Channel [a]	Strain [b]	Primer pair	Size [c] (kb)
1	MC4100 *stpA*::*cat*	P_{05}+P_{06} [d]	3.557
2	MC4100 *hns*::*kan*	P_{05}+P_{06}	2.904
3	MC4100 *stpA*::*cat hns*::*kan*	P_{05}+P_{06}	3.557
4	MC4100	P_{05}+P_{06}	2.904
M		DL 5000 marker	
1’	MC4100 *stpA*::*cat*	P_{07}+P_{08} [e]	2.913
2’	MC4100 *hns*::*kan*	P_{07}+P_{08}	3.702
3’	MC4100 *stpA*::*cat hns*::*kan*	P_{07}+P_{08}	3.702
4’	MC4100	P_{07}+P_{08}	2.913

[a] Lane numbers refer to FIG S1.

[b] Strains and PCR primers are listed in Table 1 and 2.

[c] Sizes predicted on the basis of available DNA sequence information for PCR fragments generated with primer pairs indicated in the third column.

[d] Primer pair for checking *stpA* null mutants.

[e] Primer pair for checking *hns* null mutants.

Sizes of bands detected on the agarose gels shown in FIG S1 are in good agreement with the prediction.

2. Morphology of *E. coli* mutants

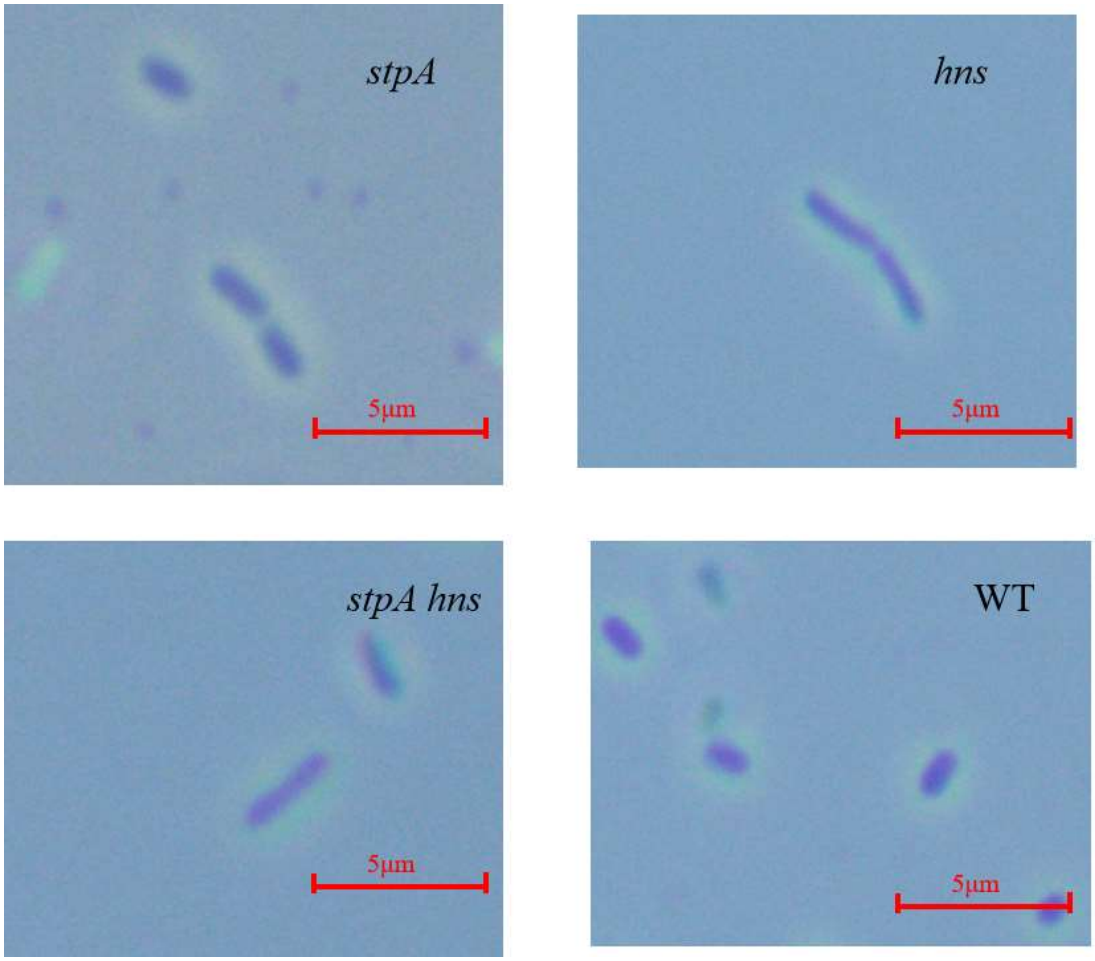
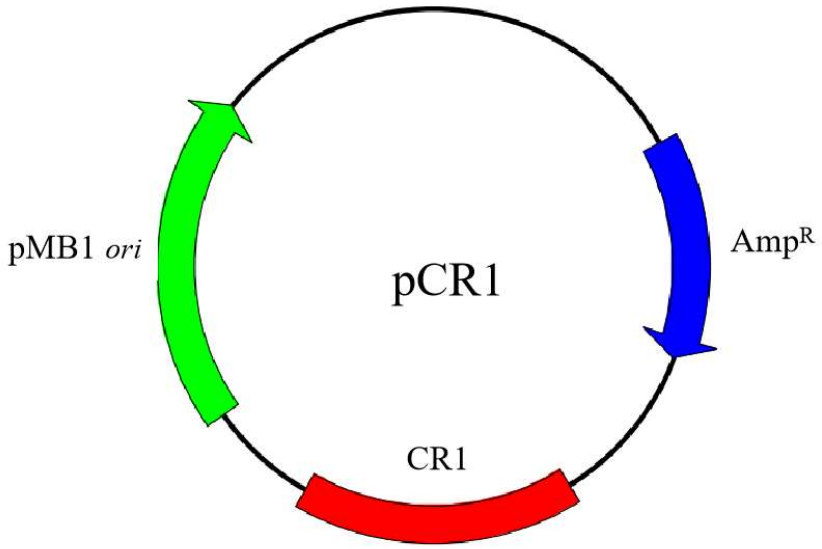


FIG S2 Morphology of constructed *hns* and *stpA* null mutants. Exponentially growing *E. coli* cells were observed with the phase-contrast microscope (1000 ×). The *hns* null mutant and the *stpA hns* null mutant were obviously longer than the *stpA* null mutant and their WT parent, in line with previous reports (7).

3. Plasmids constructed in this study

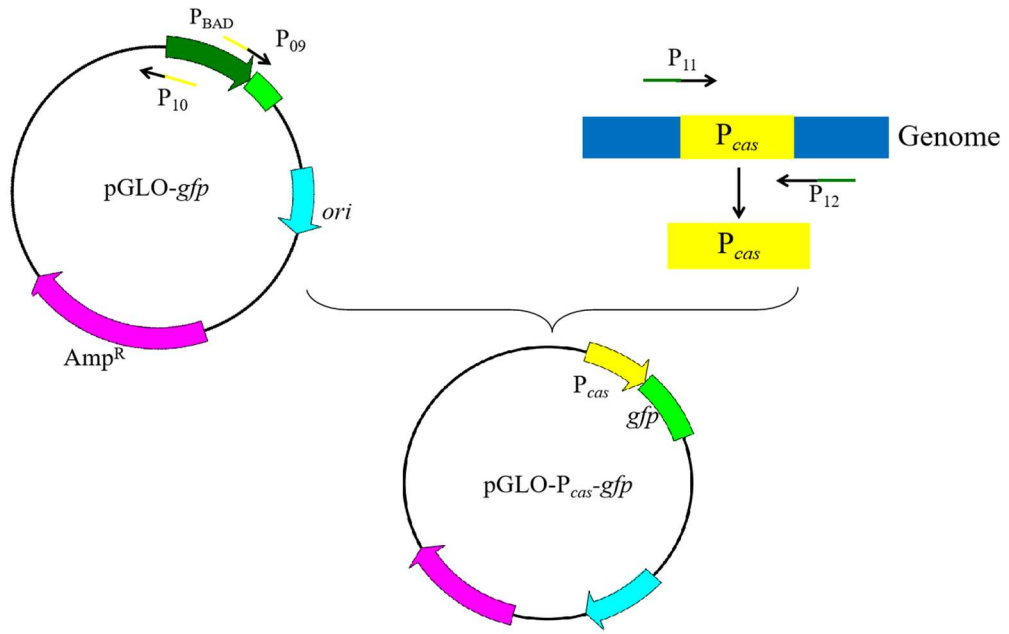


Sequences of the CR1 fragment:

5’-GAATTCGAGTTCCCCGCGCCAGCGGGGATAAAAAGCTTTCGCAGACGCG
CGGCGATACGCTCACGCAGAGTTCCCCGCGCCAGCGGGGATAAAAAGCAG
CCGAAGCCAAAGGTGATGCCGAACACGCTGAGTTCCCCGCGCCAGCGGGG
ATAAAAAGGGCTCCCTGTCGGTTGTAATTGATAATGTTGAGAGTTCCCCGC
GCCAGCGGGGATAAAAAGTTTGGATCGGGTCTGGAATTTCTGAGCGGTCGC
GAGTTCCCCGCGCCAGCGGGGATAAAAAGAAGCTT-3’

FIG S3 Schematic map and sequence of the CRISPR-Cas-targeted plasmid pCR1. PAM sequences are indicated with black letters on green background. Sequences of protospacers are indicated with black letters on red background. The complete sequence of pCR1 is available at the end of the file.

A



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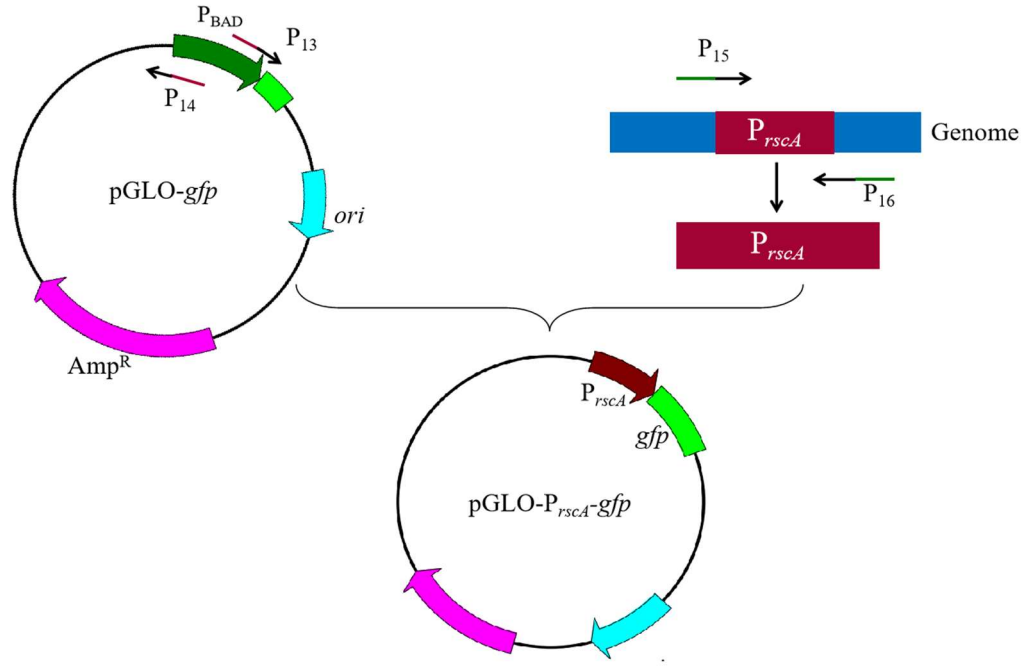

FIG S4 Construction of reporter plasmids pGLO-P*cas*-*gfp* and pGLO-P*rcsA*-*gfp*.

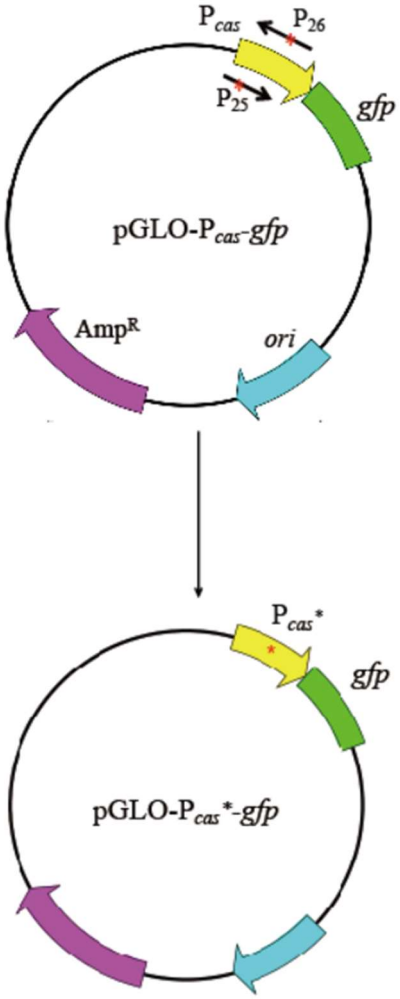

FIG S5 Construction of pGLO-P$_{cas}^{*}$-*gfp* for identifying DBS for H-NS/StpAin P$_{cas}$.

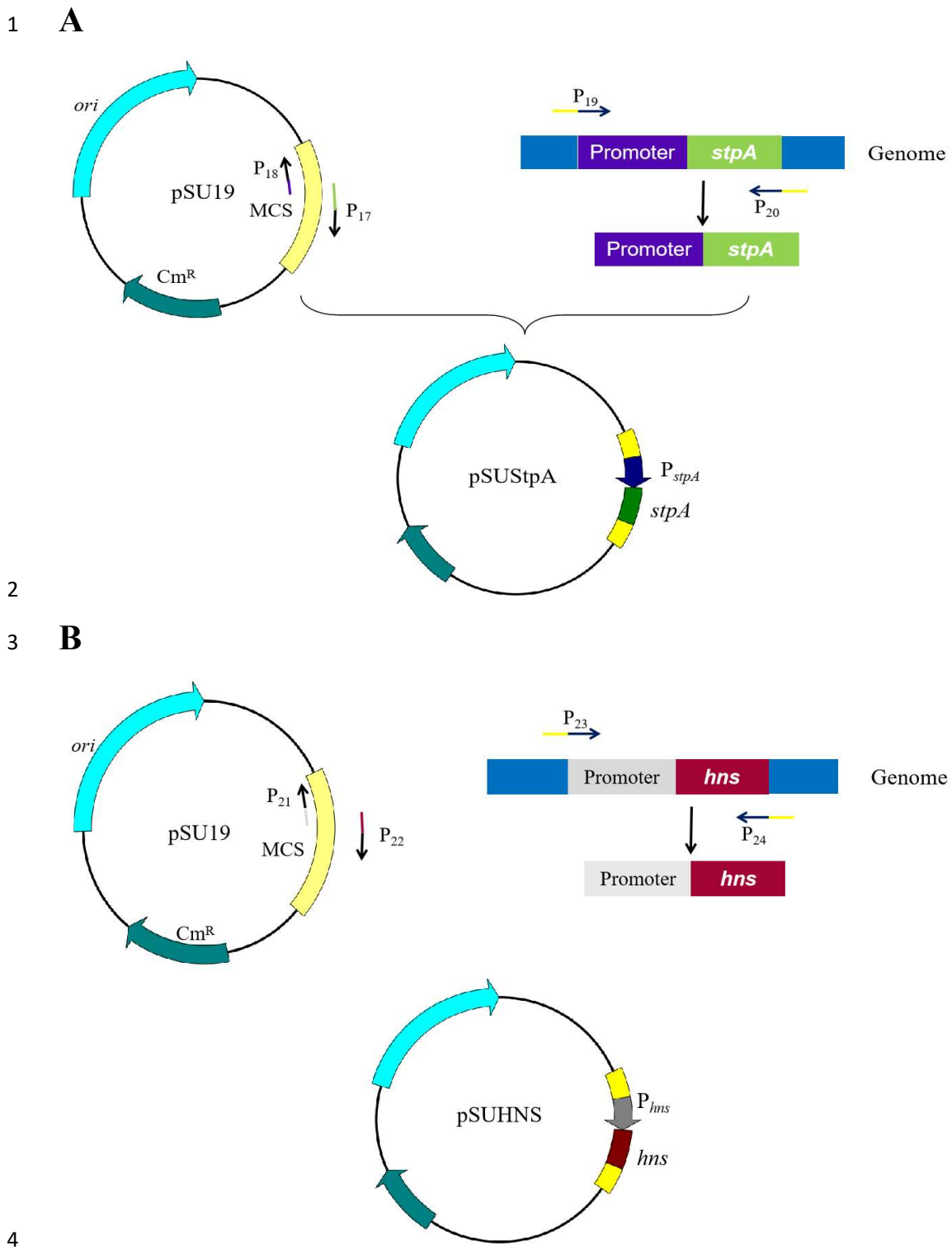

FIG S6 Construction of pSUStpA and pSUHNS.

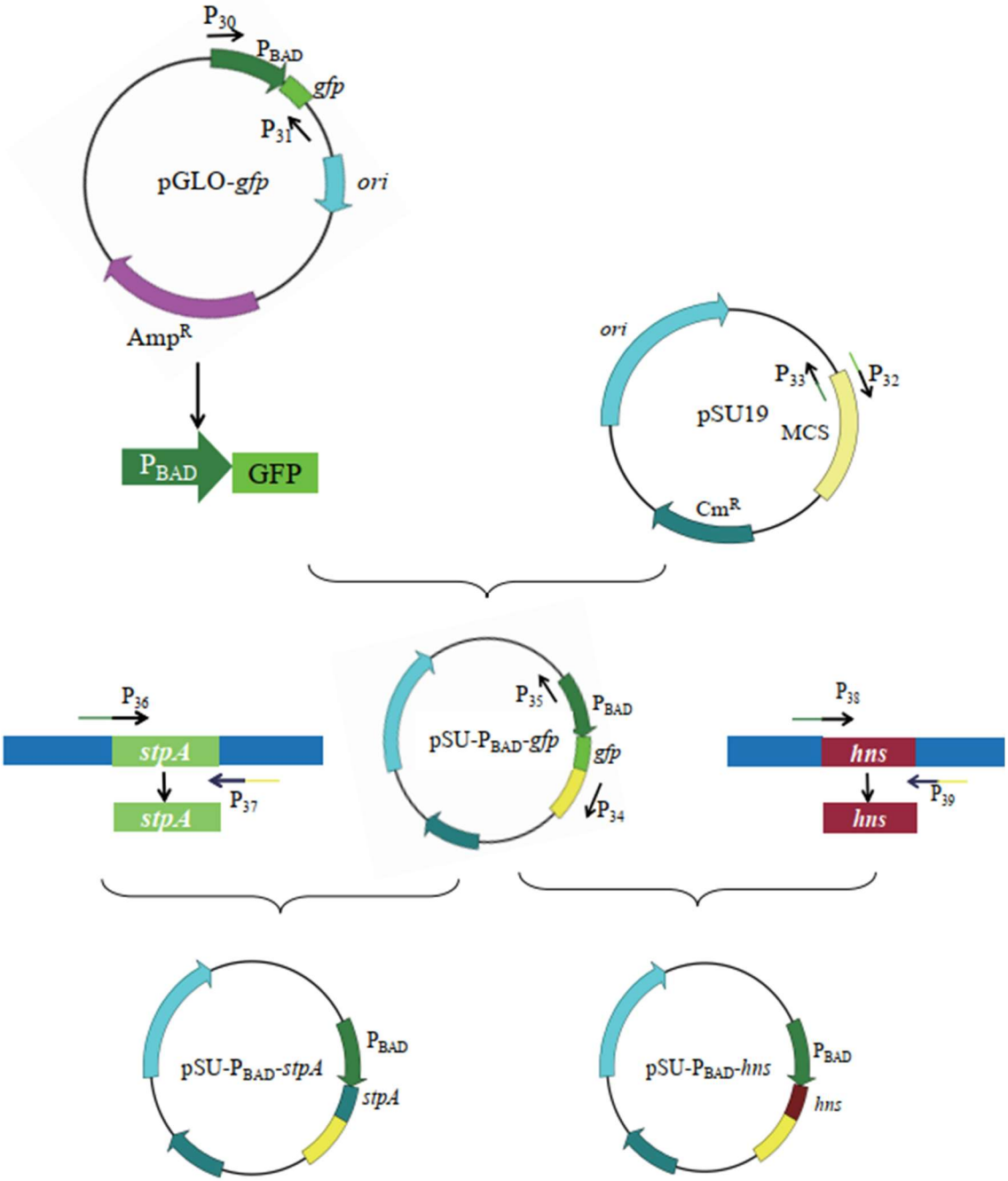

FIG S7 Construction of pSU-P$_{BAD}$-*stpA* and pSU-P$_{BAD}$-*hns*.

4. Growth of *E. coli* strains in the absence or presence of plasmid.

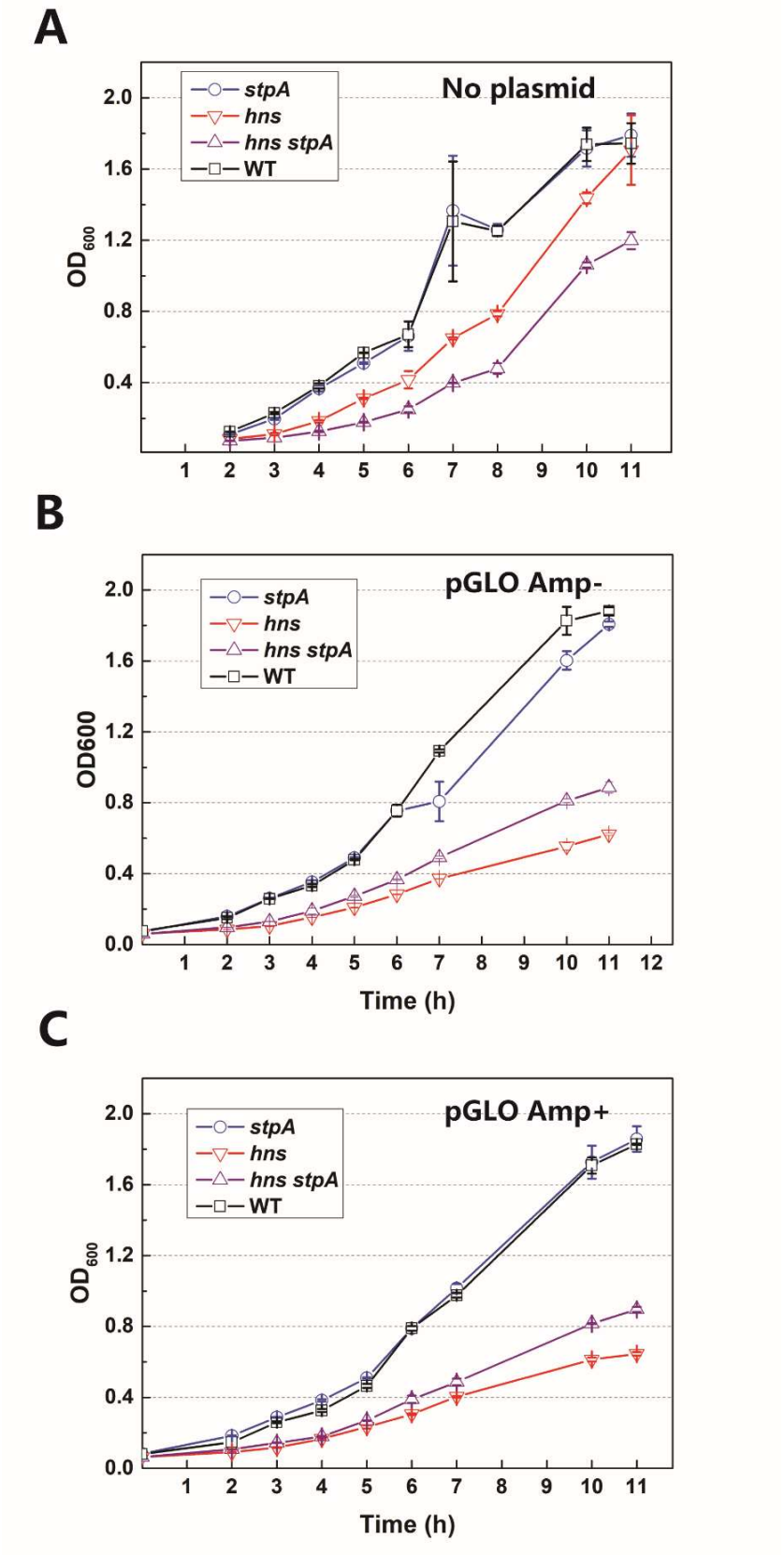

FIG S8 Plasmid affects the growth of the *hns* null mutant. In agreement with previous reports (8, 9), the *hns stpA* null mutant (purple line) grew obviously slower than the *hns* null mutant (red line) in LB broth (A). When a multi-copy-plasmid (pGLO-P_{cas}) was transferred into these strains, growths of the *stpA* mutant, the *stpA hns* null mutant and their parent were not obviously affected in LB supplemented without (B) or with (C) ampicillin (100 μg ml^{-1}). However, the growth of the *hns* null mutant carrying pGLO-P_{cas} was remarkably reduced in LB broth supplemented with or without ampicillin (B, C).

5. CRISPR-Cas-mediated DNA interference after natural transformation

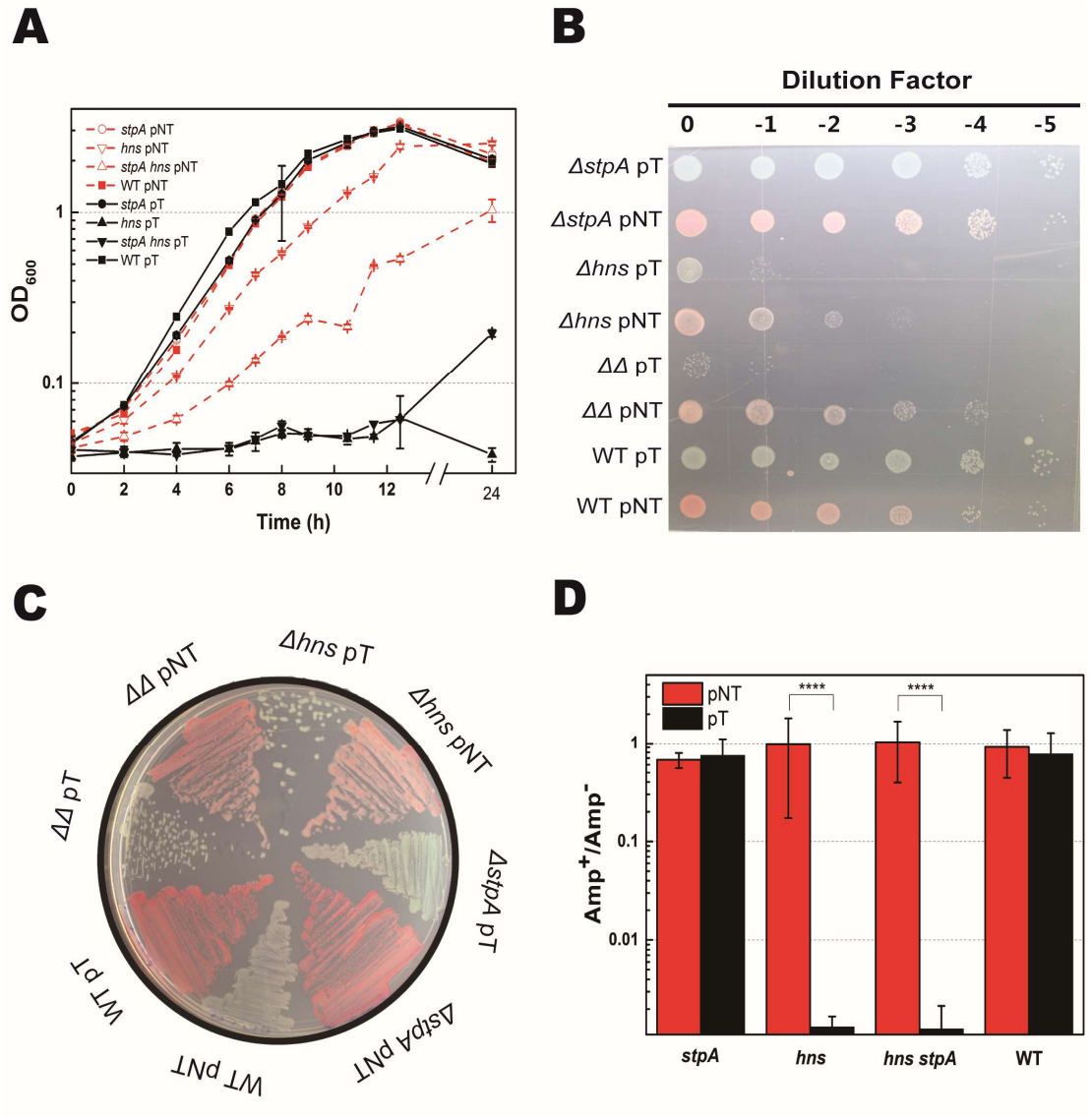

FIG S9 Plasmid loss assay in transformants. (A) Cell growths of transformants carrying the CRISPR-Cas-targeted (pT, black solid lines) and non-targeted (pNT, red dash lines) in LB broth supplemented with ampicillin (100 μg ml^{-1}). (B) Overnight-grown cell cultures with no antibiotic added were serially diluted followed by drop-plating. (C) Overnight-grown cell cultures with no antibiotic added were streaked on LB-agar plates. (D) The ratio of the number of ampicillin resistant colonies to

ampicillin sensitive colonies in the overnight-grown cell culture (no antibiotic added) was calculated. pT: pCR1; pNT: pDsRED. **** $P < 0.001$.

6. CRISPR-Cas-mediated DNA interference during chemical transformation

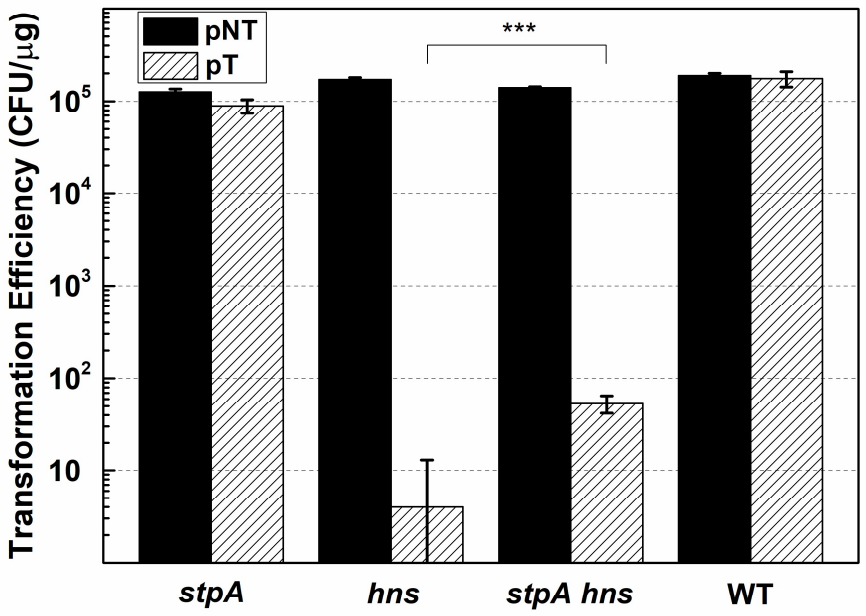

FIG S10 Effect of *stpA* inactivation on chemical transformation with CRISPR-Cas-targeted plasmid. Chemically competent cells were prepared by washing exponentially growing cells (OD_{600} 0.4 ~ 0.5) twice with the $CaCl_2$ solution (100 mM). Washed cells were then resuspended in the $CaCl_2$ solution. The competent cell suspension was mixed with plasmid on ice before a heat shock at 42 ℃ for 90 seconds. Transformed cells were recovered in LB medium and spread onto selective LB-agar plates. Levels of chemical transformation with the CRISPR-Cas-targeted plasmid pCR1 (pT) or non-targeted plasmid pDsRED (pNT) was measured in *Δhns*, *ΔstpA*, *Δhns ΔstpA* and WT. pT: pCR1; pNT: pDsRED. *** $P < 0.005$.

7. Evaluation of *E. coli* mutants and the reporter system

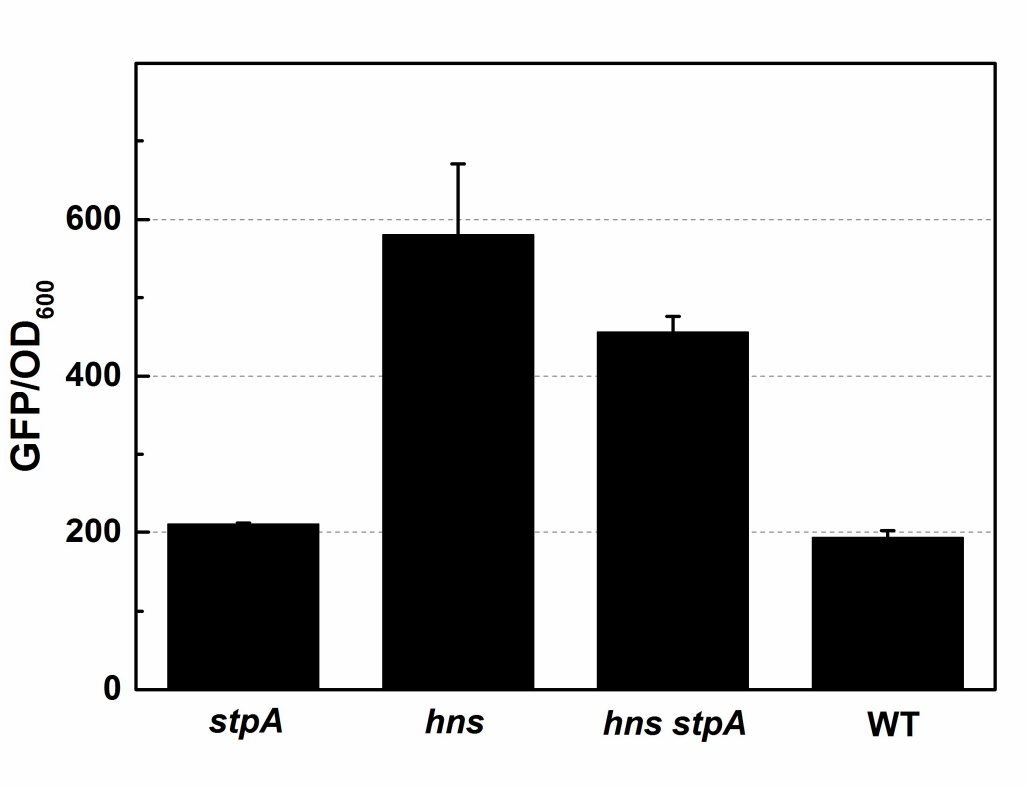

FIG S11 Quantification of transcription of *rscA* with the reporter plasmid pGLO-P$_{rcsA}$-*gfp*. By fusing the promoter P$_{rcsA}$ with *gfp*, the reporter plasmid pGLO-P$_{rcsA}$-*gfp* was constructed (FIG S4B). We transformed pGLO-P$_{rcsA}$-*gfp* into the *stpA*, *hns*, *hns stpA* null mutants and their wildtype parent. Green fluorescence was measured in the cell culture incubated in LB for 24 hours. Data are shown as mean ± SD (n = 3).

8. Expression of GFP with a constitutive promoter in *E. coli* strains

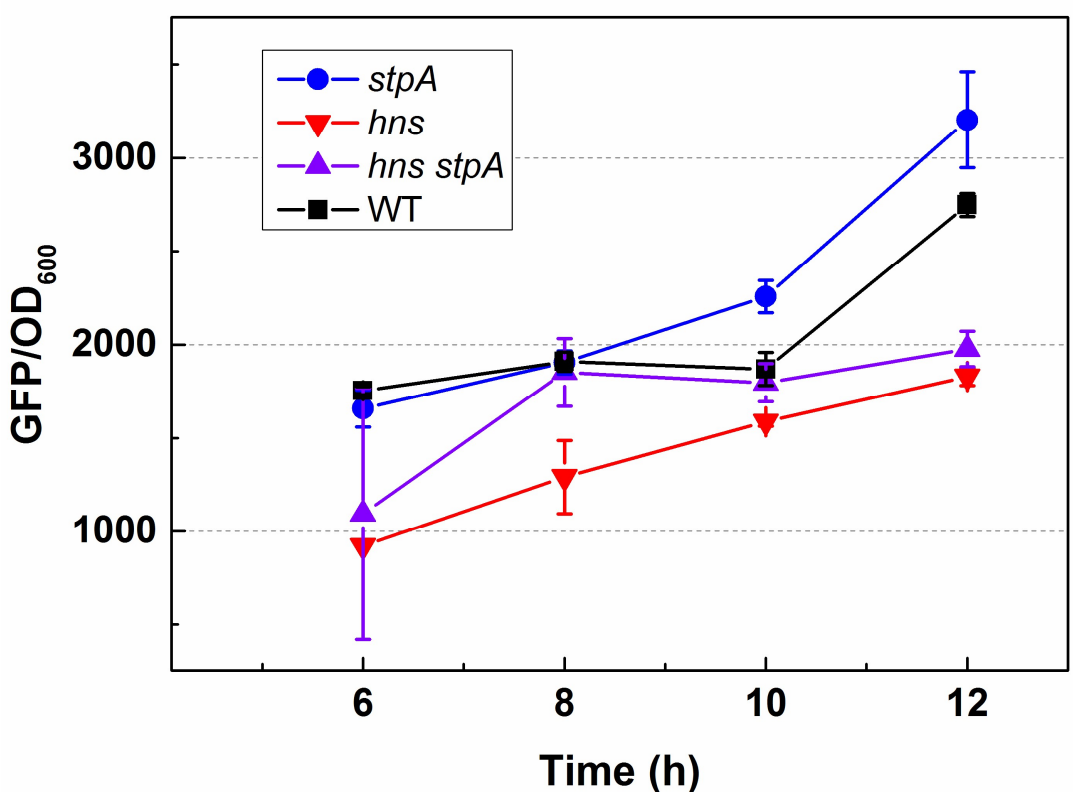

FIG S12 Expression of GFP by using P_{J23119} in the *hns* and *stpA* null mutants and their wild type parent. Expression of GFP with the constitutive promoter P_{J23119} were monitored in the *hns*, *stpA* and *hns stpA* null mutants, as well as their wildtype parent (WT). Obviously, intensities of green fluorescence in the *hns* and *hns stpA* null mutants were not higher than that in the *stpA* null mutant and WT.

9. Impact of ectopic expression of H-NS and StpA on CRISPR-Cas-mediated immunity against natural transformation during natural transformation

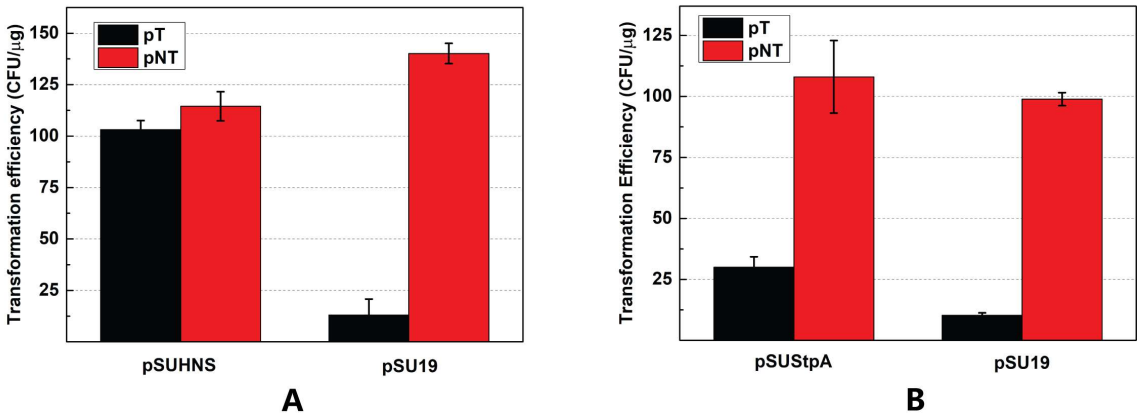

FIG S13 Effect of complementing H-NS and StpA on CRISPR-Cas-mediated DNA interference during natural transformation of the *hns stpA* mutant. Natural transformation was performed by spreading the mixture of the 24-hour incubated of *E. coli* culture and plasmid onto LB plates containing 5% (w v^{-1}) agar supplemented with ampicillin (100 μg ml^{-1}). Levels of natural transformation with the CRISPR-Cas-targeted plasmid pCR1 (pT) or non-targeted plasmid pDsRED (pNT) were measured in *Δhns ΔstpA* carrying either pSUHNS (A) or pSUStpA (B), with the empty vector as the control.

10. Effect of H-NS expression on the activity of P_{cas} in the *hns stpA* null mutant.

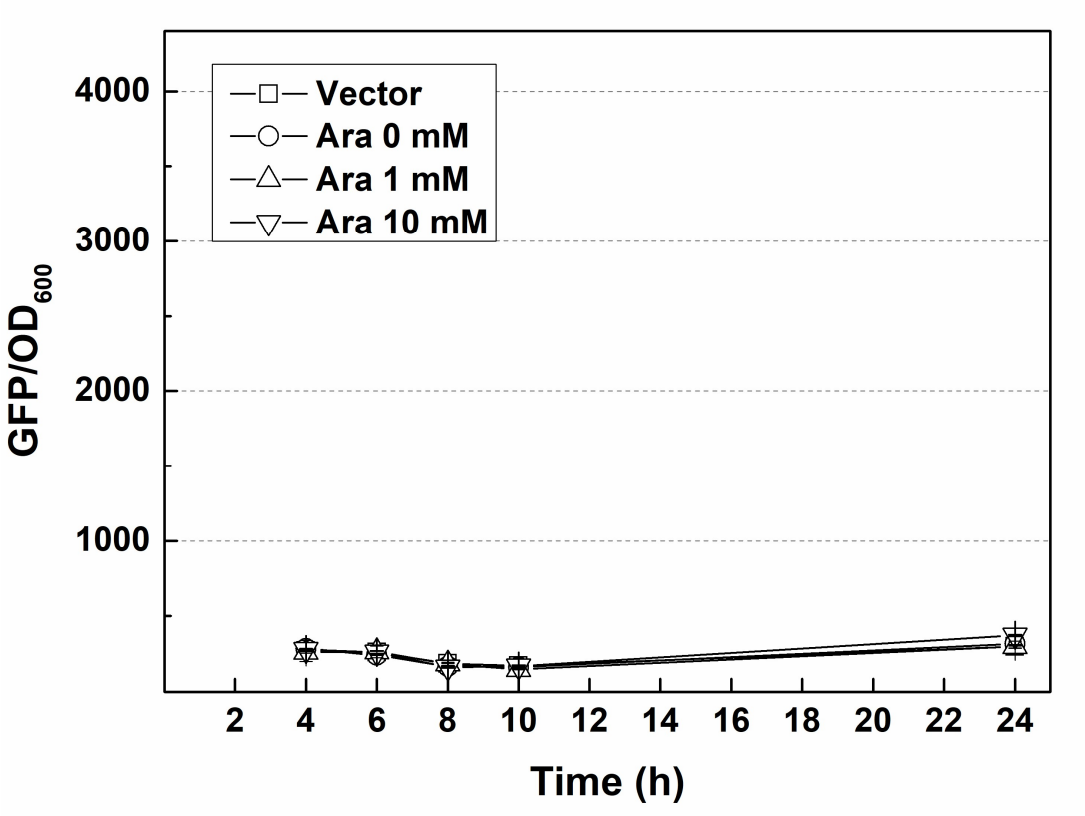

FIG S14 Expressing H-NS failed to induce transcription of the *cas* operon. StpA was found to promote the activity of P_{cas} when it was expressed at a low level in the *hns stpA* null mutant (FIG 5). To evaluate potential effect of H-NS on the activity of P_{cas} in the *hns stpA* null mutant, pSU-P_{BAD}-HNS was constructed to control the expression of H-NS with arabinose (FIG S6) with the empty vector as the control. Transcription of the *cas* operon in the *hns stpA* null mutant harboring pSU-P_{BAD}-HNS was low in M9 medium supplemented with arabinose at any tested concentrations (0 mM, 1 mM or 10 mM). Therefore, unlike StpA, expressing H-NS at low or high level failed to promote the activity of P_{cas} in the *hns stpA* null mutant.

11. Examination of the DNA binding site of H-NS in the *stpA* null mutant and its wildtype parent.

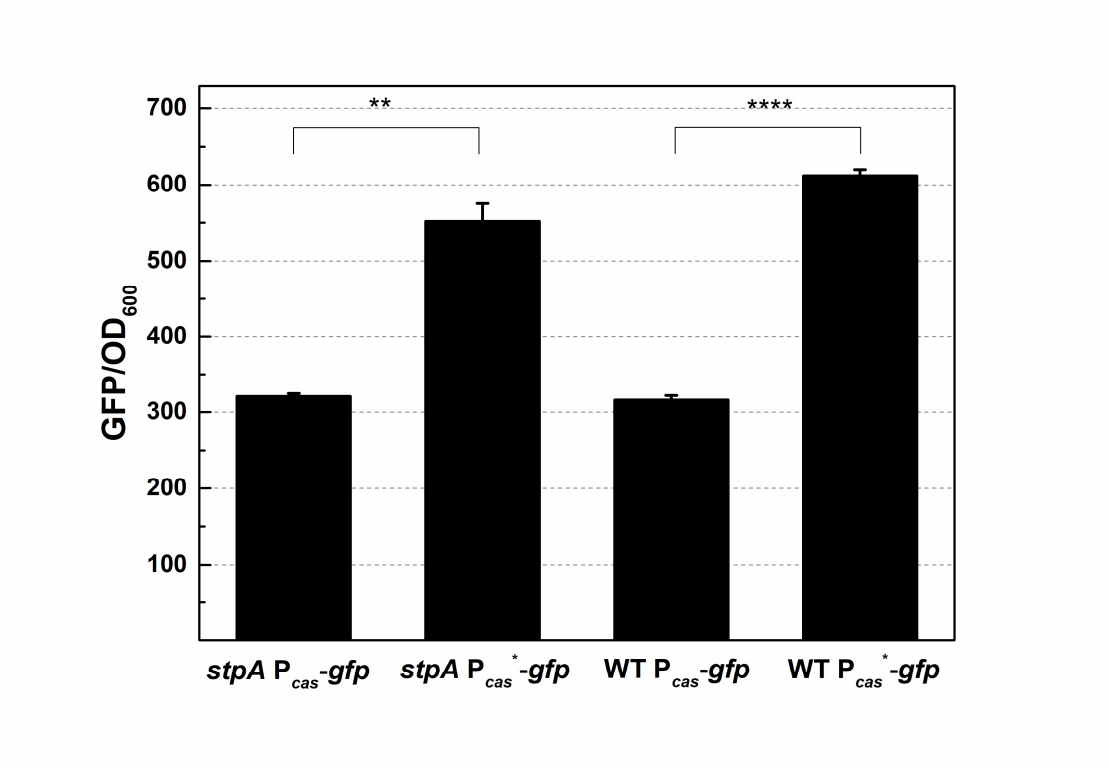

FIG S15 DNA binding site for transcriptional regulation by H-NS. Activities of P_{cas}-*gfp* and P_{cas}^{*}-*gfp* were compared in the *stpA* null mutant and its wildtype parent that were grown at 30 ℃ for 24-hour in M9 minimal medium supplemented with 0.32% (w v^{-1}) fructose. Statistical significance was determined using a two tailed Student's test. ** $P \leq 0.01$, **** $P \leq 0.001$.

12. Evaluation of qPCR-based assays for crRNA quantification

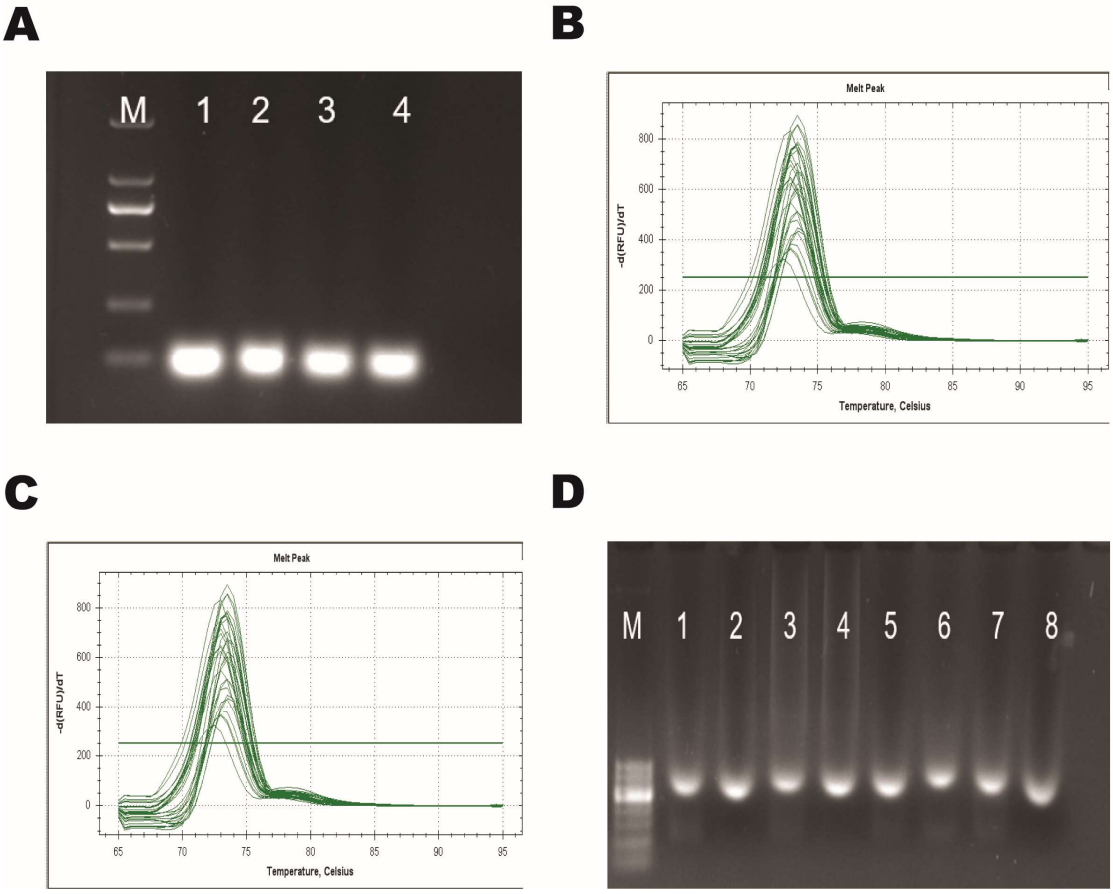

FIG S16 Examination of purified sRNA and PCR products of transcripts of crRNA and tRNA by real-time PCR. (A) Gel electrophoresis of total RNA. M: 50 bp marker; Lane 1: total sRNA from *ΔstpA*; Lane 2: total sRNA isolated from *Δhns*; Lane 3: total sRNA from *ΔstpA Δhns*; Lane 4: total sRNA from WT. (B) Melt curve of reverse transcripts of crRNA. (C) Melt curve of reverse transcripts of tRNA. (D) Gel electrophoresis of RT-PCR products. Lane 1: tRNA of *ΔstpA*; Lane 2: tRNA of *Δhns*; Lane 3: tRNA of *ΔstpA Δhns*; Lane 4: tRNA of WT; Lane 5: crRNA of *ΔstpA*; Lane 6: crRNA of *Δhns*; Lane 7: crRNA of *ΔstpA Δhns*; Lane 8: crRNA of WT; M：50 bp marker.

TABLE S2 Natural transformation of *hns stpA* pSU-P_{BAD}-*stpA* with CRISPR-targeted plasmid

Arabinose (mM)	0	0.5	1	2
TE[&] (CFU μg^{-1})	35.5 ± 7.0	5 ± 4	N. D.[*]	N. D.

[&] TE, Transformation Efficiency.

[*] N. D., Not Detected, < 1 CFU μg^{-1}

TABLE S3 Calculation of the cellular concentration of StpA

Item	Method/Reference	Result
Concentration of StpA-His	Protein assay kit	0.25 mg ml^{-1}
Concentration of StpA for WB	FIG 5F in the main text	~ 0.025 mg ml^{-1}
Amount of StpA in 1 ml culture	Proteins from 50 ml culture resuspended in 5 ml PBS	~ 0.0025 mg ml^{-1}
Viable cell density	Plate counting	~ 5.4×10^9 CFU ml^{-1}
Amount of StpA in each cell	Calculation: 0.0025 mg ml^{-1} ÷ 5.4×10^9 CFU ml^{-1}	4.6×10^{-16} g
Number of StpA in each cell	Calculation: (4.6×10^{-16} g) ÷ (15.3×10^3 Da) × (6.022×10^{23} molecules/mol)	1.8×10^4 molecules
StpA : DNA	Calculation: 1.81×10^4 molecules : 4.2×10^6 bp	~ 1 : 200

SUPPLEMENTAL REFERENCES

1. Datsenko KA, Wanner BL. 2000. One-step inactivation of chromosomal genes in *Escherichia coli* K-12 using PCR products. P Natl Acad Sci 97:6640-5.
2. Chen CY, Nace GW, Irwin PL. 2003. A 6 x 6 drop plate method for simultaneous colony counting and MPN enumeration of *Campylobacter jejuni*, *Listeria monocytogenes*, and *Escherichia coli*. J Microbiol Meth, 55:475-479.
3. Jett BD, Hatter KL, Huycke MM, Gilmore MS. 1997. Simplified agar plate method for quantifying viable bacteria. Biotechniques, 23:648-650.
4. Joseph Sambrook DWR. 2002. Molecular cloning: a laboratory manual, 3rd ed. Cold Spring Harbor Laboratory Press.
5. Sun D, Zhang X, Wang L, Prudhomme M, Xie Z, Martin B, Claverys JP. 2009. Transforming DNA uptake gene orthologs do not mediate spontaneous plasmid transformation in *Escherichia coli*. J Bacteriol 191:713-9.
6. Zhang Y, Shi C, Yu J, Ren J, Sun D. 2012. RpoS regulates a novel type of plasmid DNA transfer in *Escherichia coli*. PLoS ONE 7:e33514.
7. Kaidow A, Wachi M, Nakamura J, Magae J, Nagai K. 1995. Anucleate cell production by *Escherichia coli* delta *hns* mutant lacking a histone-like protein, H-NS. J Bacteriol 177:3589-92.
8. Zhang A, Rimsky S, Reaban ME, Buc H, Belfort M. 1996. *Escherichia coli* protein analogs StpA and H-NS: regulatory loops, similar and disparate effects on nucleic acid dynamics. EMBO J 15:1340-9.
9. Sonden B, Uhlin BE. 1996. Coordinated and differential expression of histone-like proteins in *Escherichia coli*: regulation and function of the H-NS analog StpA. EMBO J 15:4970-80.

DNA SEQUENCE INFORMATION

1. Sequence of the type I-E CRISPR-Cas system (GenBank accession number: NC_000913)

From 5' to 3':

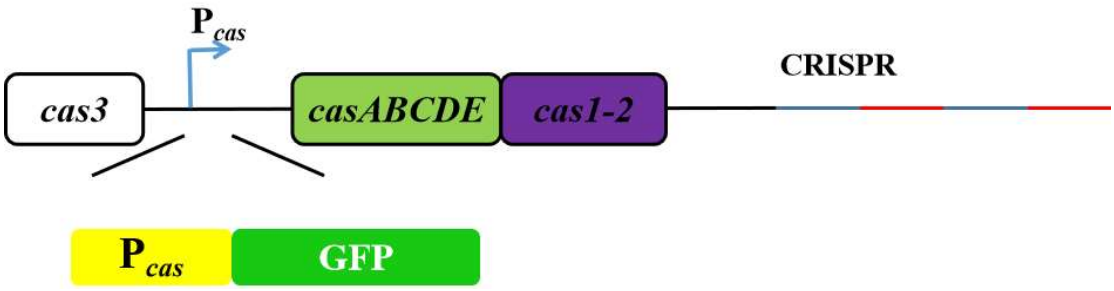

FIG S17 Sketch map of type I-E CRISPR-Cas system

The Gene encoding the nuclease

***cas3* (*ygcB*)**

ATGGAACCTTTTAAATATATATGCCATTACTGGGGAAAATCCTCAAAAAGCT
TGACGAAAGGAAATGATATTCATCTGTTAATTTATCATTGCCTTGATGTTGCT
GCTGTTGCAGATTGCTGGTGGGATCAATCAGTCGTACTGCAAAATACTTTTT
GCCGAAATGAAATGCTATCAAAACAGAGGGTGAAGGCCTGGCTGTTATTTT
TCATTGCTCTTCATGATATTGGAAAGTTTGATATACGATTCCAATATAAATCA
GCAGAAAGTTGGCTGAAATTAAATCCTGCAACGCCATCACTTAATGGTCCA
TCAACACAAATGTGCCGTAAATTTAATCATGGTGCAGCCGGTCTGTATTGGT
TTAACCAGGATTCACTTTCAGAGCAATCTCTCGGGGATTTTTTCAGTTTTTT
TGATGCCGCTCCTCATCCTTATGAGTCCTGGTTTCCATGGGTAGAGGCCGTT
ACAGGACATCATGGTTTTATATTACATTCCCAGGATCAAGATAAGTCGCGTT
GGGAAATGCCAGCTTCTCTGGCATCTTATGCTGCGCAAGATAAACAGGCTC
GTGAGGAGTGGATATCTGTACTGGAAGCATTATTTTTAACGCCAGCGGGGT
TATCTATAAACGATATACCACCTGATTGTTCATCACTGTTAGCAGGTTTTTGC
TCGCTTGCTGACTGGTTAGGCTCCTGGACTACAACGAATACCTTTCTGTTTA
ATGAGGATGCGCCTTCCGACATAAATGCTCTGAGAACGTATTTCCAGGACC
GACAGCAGGATGCGAGCCGGGTATTGGAGTTGAGTGGACTTGTATCAAATA
AGCGATGTTATGAAGGTGTTCATGCACTACTGGACAATGGCTATCAACCCA
GACAATTACAGGTGTTAGTTGATGCTCTTCCAGTAGCTCCCGGGCTGACGG
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GCCACTTATTTTCATCCCCAAATCTTATTCTTGCTCATGGCAATTCACGGTTT
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TACCTGACCTTACGATGTTAGAGCGAATGATCGCAGCGGCAAACGCGGGTG
CACAGGTCTGTCTTATTTGCAATTTGGTTGACGTTGCACAAGTATGCTACCA
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GCGCTTTACGCTGAACGATCGTCGTGAAAAAGAGAATCGAGTTATTAGCAA
TTTCGGCAAAAATGGGAAGCGAAATGTTGGACGGATACTTGTCGCAACCC
AGGTCGTGGAACAATCACTCGACGTTGATTTTGATTGGTTAATTACTCAGCA
TTGTCCTGCAGATTTGCTTTTCCAACGATTGGGCCGTTTACATCGCCATCAT
CGCAAATATCGTCCCGCTGGTTTTGAGATTCCTGTTGCCACCATTTTGCTGC
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TCATGTGGCGGACGCAGCAACATATTGAGGAGCTTAATGGAGCATCCTTATT
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GGGAAATGAGCCTGCCATTATTGCCTTATGTACAAACGTCTTCAGGTAAAC
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ATCTGGATGGATGGGTCTGGCAGGGTAACAGTATTGTTATTACCTATACAGG
GGATGAAGGGATGACCAGAGTCATCCCTGCAAATCCCAAATAA

Genes encoding components of Cascade

casA (*ygcL*)

ATGAATTTGCTTATTGATAACTGGATCCCTGTACGCCCGCGAAACGGGGGG
AAAGTCCAAATCATAAATCTGCAATCGCTATACTGCAGTAGAGATCAGTGG
CGATTAAGTTTGCCCCGTGACGATATGGAACTGGCCGCTTTAGCACTGCTG
GTTTGCATTGGGCAAATTATCGCCCCGGCAAAAGATGACGTTGAATTTCGA
CATCGCATAATGAATCCGCTCACTGAAGATGAGTTTCAACAACTCATCGCG
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CCAAAGGTGTCAAAGCAAATGATGTGACTCCAATGGAAAAACTGTTGGCT
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GCTTGCCCGCGCCACGCTATACAAACATTTACGGGAGTTAAAACCGCAAGG
AGGGCCATCAAATGGCTGA

***casB* (*ygcK*)**

ATGGCTGATGAAATTGATGCAATGGCTTTATATCGAGCCTGGCAACAACTGG
ATAATGGATCATGTGCGCAAATTAGACGTGTTTCAGAACCTGATGAATTACG
CGATATCCCTGCGTTTTATAGGCTGGTGCAACCTTTTGGTTGGGAAAACCCA
CGTCACCAGCAGGCTCTTTTGCGCATGGTGTTTTGCCTGAGCGCAGGAAAG
AATGTCATCCGACATCAGGACAAAAAATCGGAGCAAACAACAGGTATCTC
GTTGGGAAGAGCTTTAGCCAATAGTGGAAGAATTAACGAGCGCCGTATCTT
TCAATTAATTCGGGCTGACAGAACAGCCGATATGGTCCAGTTACGTCGATTA
CTTACTCACGCCGAACCCGTACTTGACTGGCCATTAATGGCCAGGATGTTG
ACCTGGTGGGGAAAGCGCGAACGCCAGCAACTTCTGGAAGATTTTGTATT
GACCACAAACAAAAATGCGTAA

***casC* (*ygcJ*)**

ATGTCTAACTTTATCAATATTCATGTTCTGATCTCTCACAGCCCTTCATGTCT
GAACCGCGACGATATGAACATGCAGAAAGACGCTATTTTCGGCGGCAAAA
GACGAGTAAGAATTTCAAGTCAAAGCCTTAAACGTGCGATGCGTAAAAGT
GGTTATTACGCACAAAATATTGGTGAATCCAGTCTCAGAACCATTCATCTTG
CACAATTACGTGATGTTCTTCGGCAAAAACTTGGTGAACGTTTTGACCAAA
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AGTTAAACAAATGCCTACTTTAGAACAGTTAAAATCCTGGGTTCGTAATAAT
GGCGAGGCGTGA

***casD* (*ygcI*)**

GTGAACATGAGATCTTATTTGATCTTGCGGCTTGCTGGGCCAATGCAAGCCT
GGGGGCAGCCGACCTTTGAAGGAACGCGACCTACCGGAAGATTTCCGACC
CGAAGCGGGTTATTAGGGCTACTCGGGGCTTGTCTTGGGATCCAACGTGAT
GATACTTCTTCATTACAGGCGTTATCAGAGAGTGTGCAATTTGCAGTGCGCT
GCGATGAACTCATTCTTGACGATCGTCGTGTGTCTGTAACGGGGTTGCGTG
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GGCGCTATTAAATTATGAGCCCGTTGGCGGCGATATATATAGTGAGGAATCA
GTTACAGGGCATCATTTAAAATTTACGGCGCGCGACGAACCGATGATCACC
TTGCCTCGACAATTTGCTTCCCGAGAATGGTATGTGATTAAAGGAGGTATGG
ATGTATCTCAGTAA

***casE* (*ygcH*)**

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TGATTTTCTTTTTCATGTTGAGAAGCGAAACACACCAGAAGGCTGTCATGT
TTTATTGCAGTCAGCGCAAATGCCTGTTTCAACTGCCGTTGCGACAGTCATT
AAAACTAAACAGGTTGAATTTCAACTTCAGGTTGGTGTTCCACTCTATTTTC
GGCTTCGGGCAAATCCGATCAAAACTATTCTCGACAATCAAAAGCGCCTGG
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AACAAATCGCGTGGTTGCAACGTAAATTGGGCAATGCGGCGCGCGTTGAA
GATGTGCATCCCATATCGGAACGGCCACAGTATTTTTCTGGTGATGGTAAAA
GTGGAAAGATCCAAACGGTTTGCTTTGAAGGTGTGCTCACCATCAACGAC
GCGCCAGCGTTAATAGATCTTGTACAGCAAGGTATTGGGCCAGCTAAATCG
ATGGGATGTGGCTTGCTATCTTTGGCTCCACTGTGA

Genes encoding Cas1 and Cas2 which are involved in adaption

***cas1* (*ygbT*)**

ATGACCTGGCTTCCCCTTAATCCCATTCCACTCAAAGATCGCGTCTCCATGA
TCTTTCTGCAATATGGGCAGATCGATGTAATAGATGGCGCGTTTGTACTTATC
GACAAGACAGGGATCCGCACTCATATTCCTGTTGGCTCGGTTGCCTGCATC
ATGCTGGAACCTGGTACACGGGTTTCGCATGCAGCTGTACGCCTGGCTGCG
CAAGTTGGAACATTGTTGGTATGGGTGGGGGAAGCGGGCGTTCGTGTTTAT
GCTTCTGGTCAGCCTGGAGGTGCGCGTTCAGATAAGCTGCTCTATCAGGCA
AAACTTGCTCTGGATGAAGATTTGCGTCTGAAGGTCGTACGTAAAATGTTT
GAACTTCGGTTTGGAGAACCTGCGCCTGCCCGGCGCTCCGTAGAGCAACT
CAGAGGTATAGAAGGCAGTCGCGTGCGGGCAACCTACGCACTTCTGGCGA
AGCAATACGGCGTGACATGGAATGGACGTCGCTACGATCCGAAAGACTGG
GAAAAGGGCGATACGATCAACCAATGCATTAGCGCTGCAACTTCCTGTTTA
TACGGCGTAACTGAAGCGGCGATACTTGCAGCTGGTTATGCACCAGCTATT
GGGTTTGTGCATACAGGAAAGCCTCTTTCCTTTGTTTACGATATTGCAGACA
TCATTAAATTTGACACTGTTGTACCGAAAGCTTTTGAGATAGCGCGTCGTAA
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GCTGGAGAAATACAACCGCCGGCCCCACCTGAAGATGCACAGCCTGTTGC
CATTCCGCTTCCTGTTTCACTGGGAGATGCAGGCCATCGGAGTAGCTGA

***cas2* (*ygbF*)**

ATGAGTATGTTGGTCGTGGTCACTGAAAATGTACCTCCGCGCTTACGAGGC
AGATTAGCCATCTGGTTGTTGGAGGTACGTGCAGGGGTATATGTAGGTGATG
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AAGAAGGCAATGTAGTGATGGCATGGGCAACGAATACGGAAACGGGATTT
GAGTTCCAGACATTTGGGTTAAACAGGCGTACCCCGGTAGATTTGGATGGT
TTAAGGTTGGTGTCTTTTTTACCTGTTTGA

CRISPR array I (Repeats are indicated with black letters on yellow background. Spacers are indicated with black letters on green background)

From 5' to 3':

TGGGAACAAATAGCTGGACTGGCGGAAGAAGGCAATGTAGTGATGGCATG
GGCAACGAATACGGAAACGGGATTTGAGTTCCAGACATTTGGGTTAAACA
GGCGTACCCCGGTAGATTTGGATGGTTTAAGGTTGGTGTCTTTTTTACCTGT
TTGAAAACAAAGAATTAGCTGATCTTTAATAATAAGGAAATGTTACATTAAG
GTTGGTGGGTTGTTTTTATGGGAAAAAATGCTTTAAGAACAAATGTATACTT
TTAGAGAGTTCCCCGCGCCAGCGGGGATAAACCGCTTTCGCAGACGCGCG
GCGATACGCTCACGCAGAGTTCCCCGCGCCAGCGGGGATAAACCGCAGCC
GAAGCCAAAGGTGATGCCGAACACGCTGAGTTCCCCGCGCCAGCGGGGAT
AAACCGGGCTCCCTGTCGGTTGTAATTGATAATGTTGAGAGTTCCCCGCGC
CAGCGGGGATAAACCGTTTGGATCGGGTCTGGAATTTCTGAGCGGTCGCGA
GTTCCCCGCGCCAGCGGGGATAAACCGCGAATCGCGCATACCCTGCGCGTC

GCCGCCTGCGAGTTCCCCGCGCCAGCGGGGATAAACCGTCAGCTTTATAAA
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CTCACCCCGAAAGAGATTGCCAGCCAGCTTGAGTTCCCCGCGCCAGCGGG
GATAAACCGCTGCTGGAGCTGGCTGCAAGGCAAGCCGCCCAGAGTTCCCC
GCGCCAGCGGGGATAAACCGGGGGGCGCATGACCGTAAACATTATCCCCCG
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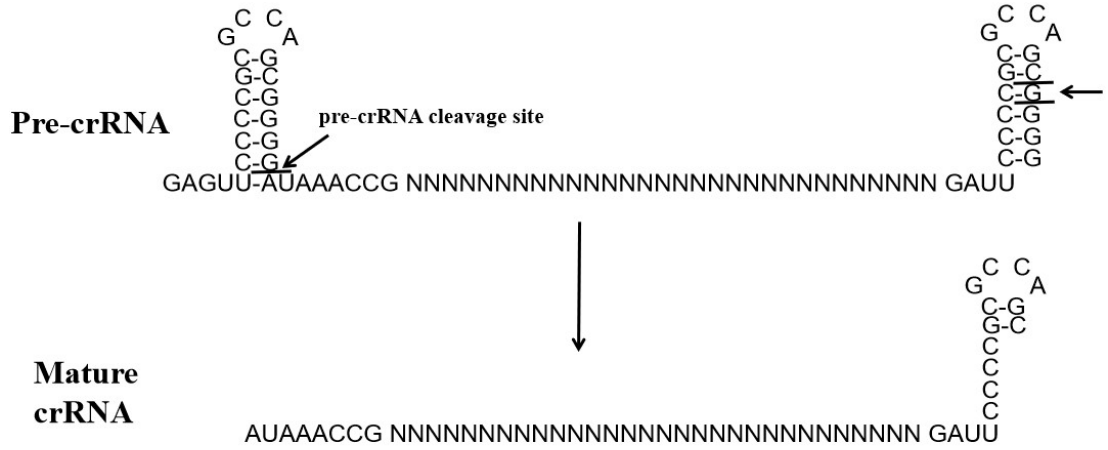
CCCGCCTCTGCGGTAGAACTCCCAGCTCCCATTTTCAAACCCATCAAGACG
CCTTCGCCAACTCCTTCACCAGAGGTAGCATTATCCGCATA

2. Sequence of the mature crRNA (N represents A/U/C/G in the spacer region)

From 5' to 3':



3. Sequence of the promoter of the *cas* operon (GenBank accession number: NC_000913; TSS: Transcription Start Site)

From 5' to 3':

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-350

GGGAA GGTGC ACAAT GTACA TCTTC TTTTA ATTTC CCGGT ATGAG

-300

ATTTT ATATT CACAG TATGA ATATTT TATGTA ATAAA ATTCAT GGTAA

-250 **H-NS Binding Site**

TTATT ATAAC TAAAA GTTTC TTT**AA TAATA AAACG AA**TAA CTTGC

-200 **TSS**

AGATT TGAAA TGCAT GCATT ATTGT CTTTA A**A**CAA TTCAA CACAT

-150

CTTAA TATAT GTATA GGTTA ATTGT ATTAA ACCAA TGAAT ATATT TTTGC

-100

AGTGA ATGTG ATTAT TGAAT TAATT ACGCC GTATT TTTTC TTTGT TTTTA

-50

CCGAT AACGG AAGTG TGCCG ACGTA TAGAA ATGCA GGAGA AATGT

-1

CGGAG CATAT GAAGG AGAACAA

4. Complete Sequence of pCR1 (AddGene accession number: 154270; repeats and PAMs are on red and green backgrounds respectively)

From 5' to 3':

TCGCGCGTTTCGGTGATGACGGTGAAAACCTCTGACACATGCAGCTCCCGG
AGACGGTCACAGCTTGTCTGTAAGCGGATGCCGGGAGCAGACAAGCCCGT
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GTTCCCCGCGCCAGCGGGGATAAAAAGCTTTCGCAGACGCGCGGCGATAC
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AAGGTGATGCCGAACACGCTGAGTTCCCCGCGCCAGCGGGGATAAAAAGG
GCTCCCTGTCGGTTGTAATTGATAATGTTGAGAGTTCCCCGCGCCAGCGGG
GATAAAAAGTTTGGATCGGGTCTGGAATTTCTGAGCGGTCGCGAGTTCCCC
GCGCCAGCGGGGATAAAAAGAAGCTTAGCTTGGCGTAATCATGGTCATAGC
TGTTTCCTGTGTGAAATTGTTATCCGCTCACAATTCCACACAACATACGAGC
CGGAAGCATAAAGTGTAAAGCCTGGGGTGCCTAATGAGTGAGCTAACTCA
CATTAATTGCGTTGCGCTCACTGCCCGCTTTCCAGTCGGGAAACCTGTCGT
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CAGAATCAGGGGATAACGCAGGAAAGAACATGTGAGCAAAAGGCCAGCA
AAAGGCCAGGAACCGTAAAAAGGCCGCGTTGCTGGCGTTTTTCCATAGGC
TCCGCCCCCCTGACGAGCATCACAAAAATCGACGCTCAAGTCAGAGGTGG
CGAAACCCGACAGGACTATAAAGATACCAGGCGTTTCCCCCTGGAAGCTCC
CTCGTGCGCTCTCCTGTTCCGACCCTGCCGCTTACCGGATACCTGTCCGCCT
TTCTCCCTTCGGGAAGCGTGGCGCTTTCTCATAGCTCACGCTGTAGGTATCT
CAGTTCGGTGTAGGTCGTTCGCTCCAAGCTGGGCTGTGTGCACGAACCCCC
CGTTCAGCCCGACCGCTGCGCCTTATCCGGTAACTATCGTCTTGAGTCCAAC
CCGGTAAGACACGACTTATCGCCACTGGCAGCAGCCACTGGTAACAGGATT
AGCAGAGCGAGGTATGTAGGCGGTGCTACAGAGTTCTTGAAGTGGTGGCC
TAACTACGGCTACACTAGAAGAACAGTATTTGGTATCTGCGCTCTGCTGAA
GCCAGTTACCTTCGGAAAAAGAGTTGGTAGCTCTTGATCCGGCAAACAAA

CCACCGCTGGTAGCGGTGGTTTTTTTGTTTGCAAGCAGCAGATTACGCGCA
GAAAAAAAGGATCTCAAGAAGATCCTTTGATCTTTTCTACGGGGTCTGACG
CTCAGTGGAACGAAAACTCACGTTAAGGGATTTTGGTCATGAGATTATCAA
AAAGGATCTTCACCTAGATCCTTTTAAATTAAAAATGAAGTTTTAAATCAAT
CTAAAGTATATATGAGTAAACTTGGTCTGACAGTTACCAATGCTTAATCAGT
GAGGCACCTATCTCAGCGATCTGTCTATTTCGTTCATCCATAGTTGCCTGAC
TCCCCGTCGTGTAGATAACTACGATACGGGAGGGCTTACCATCTGGCCCCA
GTGCTGCAATGATACCGCGAGACCCACGCTCACCGGCTCCAGATTTATCAG
CAATAAACCAGCCAGCCGGAAGGGCCGAGCGCAGAAGTGGTCCTGCAACT
TTATCCGCCTCCATCCAGTCTATTAATTGTTGCCGGGAAGCTAGAGTAAGTA
GTTCGCCAGTTAATAGTTTGCGCAACGTTGTTGCCATTGCTACAGGCATCGT
GGTGTCACGCTCGTCGTTTGGTATGGCTTCATTCAGCTCCGGTTCCCAACG
ATCAAGGCGAGTTACATGATCCCCCATGTTGTGCAAAAAAGCGGTTAGCTC
CTTCGGTCCTCCGATCGTTGTCAGAAGTAAGTTGGCCGCAGTGTTATCACT
CATGGTTATGGCAGCACTGCATAATTCTCTTACTGTCATGCCATCCGTAAGAT
GCTTTTCTGTGACTGGTGAGTACTCAACCAAGTCATTCTGAGAATAGTGTAT
GCGGCGACCGAGTTGCTCTTGCCCGGCGTCAATACGGGATAATACCGCGCC
ACATAGCAGAACTTTAAAAGTGCTCATCATTGGAAAACGTTCTTCGGGGCG
AAAACTCTCAAGGATCTTACCGCTGTTGAGATCCAGTTCGATGTAACCCAC
TCGTGCACCCAACTGATCTTCAGCATCTTTTACTTTCACCAGCGTTTCTGGG
TGAGCAAAAACAGGAAGGCAAAATGCCGCAAAAAAGGGAATAAGGGCGA
CACGGAAATGTTGAATACTCATACTCTTCCTTTTTCAATATTATTGAAGCATT
TATCAGGGTTATTGTCTCATGAGCGGATACATATTTGAATGTATTTAGAAAAA
TAAACAAATAGGGGTTCCGCGCACATTTCCCCGAAAAGTGCCACCTGACGT
CTAAGAAACCATTATTATCATGACATTAACCTATAAAAATAGGCGTATCACG
AGGCCCTTTCGTC

5. Complete Sequence of pDsRed (sequence of *rfp* marked on red background)

Available in the website: http://www.biofeng.com/zaiti/dachang/pDsRed-Express2.html

From 5' to 3':

AGCGCCCAATACGCAAACCGCCTCTCCCCGCGCGTTGGCCGATTCATTAAT
GCAGCTGGCACGACAGGTTTCCCGACTGGAAAGCGGGCAGTGAGCGCAA
CGCAATTAATGTGAGTTAGCTCACTCATTAGGCACCCCAGGCTTTACACTTT
ATGCTTCCGGCTCGTATGTTGTGTGGAATTGTGAGCGGATAACAATTTCACA
CAGGAAACAGCTATGACCATGATTACGCCAAGCTTGCATGCCTGCAGGTCG
ACTCTAGAGGATCCCCGGGTACCGGTCGCCACCATGGATAGCACTGAGAAC
GTCATCAAGCCCTTCATGCGCTTCAAGGTGCACATGGAGGGCTCCGTGAAC
GGCCACGAGTTCGAGATCGAGGGCGAGGGCGAGGGCAAGCCCTACGAGG
GCACCCAGACCGCCAAGCTGCAGGTGACCAAGGGCGGCCCCCTGCCCTTC
GCCTGGGACATCCTGTCCCCCCAGTTCCAGTACGGCTCCAAGGTGTACGTG
AAGCACCCCGCCGACATCCCCGACTACAAGAAGCTGTCCTTCCCCGAGGG
CTTCAAGTGGGAGCGCGTGATGAACTTCGAGGACGGCGGCGTGGTGACCG
TGACCCAGGACTCCTCCCTGCAGGACGGCACCTTCATCTACCACGTGAAGT
TCATCGGCGTGAACTTCCCCTCCGACGGCCCCGTAATGCAGAAGAAGACTC
TGGGCTGGGAGCCCTCCACCGAGCGCCTGTACCCCCGCGACGGCGTGCTG
AAGGGCGAGATCCACAAGGCGCTGAAGCTGAAGGGCGGCGGCCACTACCT
GGTGGAGTTCAAGTCAATCTACATGGCCAAGAAGCCCGTGAAGCTGCCCG
GCTACTACTACGTGGACTCCAAGCTGGACATCACCTCCCACAACGAGGACT
ACACCGTGGTGGAGCAGTACGAGCGCGCCGAGGCCCGCCACCACCTGTTC
CAGTAGCGGCCGCGACTCTAGAATTCCAACTGAGCGCCGGTCGCTACCATT
ACCAACTTGTCTGGTGTCAAAAATAATAGGCCTACTAGTCGGCCGTACGGG
CCCTTTCGTCTCGCGCGTTTCGGTGATGACGGTGAAAACCTCTGACACATG
CAGCTCCCGGAGACGGTCACAGCTTGTCTGTAAGCGGATGCCGGGAGCAG
ACAAGCCCGTCAGGGCGCGTCAGCGGGTGTTGGCGGGTGTCGGGGCTGGC
TTAACTATGCGGCATCAGAGCAGATTGTACTGAGAGTGCACCATATGCGGT
GTGAAATACCGCACAGATGCGTAAGGAGAAAATACCGCATCAGGCGGCCTT
AAGGGCCTCGTGATACGCCTATTTTTATAGGTTAATGTCATGATAATAATGGT
TTCTTAGACGTCAGGTGGCACTTTTCGGGGAAATGTGCGCGGAACCCCTAT
TTGTTTATTTTTCTAAATACATTCAAATATGTATCCGCTCATGAGACAATAAC
CCTGATAAATGCTTCAATAATATTGAAAAAGGAAGAGTATGAGTATTCAACA
TTTCCGTGTCGCCCTTATTCCCTTTTTTGCGGCATTTTGCCTTCCTGTTTTTG
CTCACCCAGAAACGCTGGTGAAAGTAAAAGATGCTGAAGATCAGTTGGGT
GCACGAGTGGGTTACATCGAACTGGATCTCAACAGCGGTAAGATCCTTGAG
AGTTTTCGCCCCGAAGAACGTTTTCCAATGATGAGCACTTTTAAAGTTCTG
CTATGTGGCGCGGTATTATCCCGTATTGACGCCGGGCAAGAGCAACTCGGT
CGCCGCATACACTATTCTCAGAATGACTTGGTTGAGTACTCACCAGTCACA
GAAAAGCATCTTACGGATGGCATGACAGTAAGAGAATTATGCAGTGCTGCC
ATAACCATGAGTGATAACACTGCGGCCAACTTACTTCTGACAACGATCGGA
GGACCGAAGGAGCTAACCGCTTTTTTGCACAACATGGGGGATCATGTAACT

CGCCTTGATCGTTGGGAACCGGAGCTGAATGAAGCCATACCAAACGACGA
GCGTGACACCACGATGCCTGTAGCAATGGCAACAACGTTGCGCAAACTATT
AACTGGCGAACTACTTACTCTAGCTTCCCGGCAACAATTAATAGACTGGAT
GGAGGCGGATAAAGTTGCAGGACCACTTCTGCGCTCGGCCCTTCCGGCTG
GCTGGTTTATTGCTGATAAATCTGGAGCCGGTGAGCGTGGGTCTCGCGGTAT
CATTGCAGCACTGGGGCCAGATGGTAAGCCCTCCCGTATCGTAGTTATCTAC
ACGACGGGGAGTCAGGCAACTATGGATGAACGAAATAGACAGATCGCTGA
GATAGGTGCCTCACTGATTAAGCATTGGTAACTGTCAGACCAAGTTTACTC
ATATATACTTTAGATTGATTTAAAACTTCATTTTTAATTTAAAAGGATCTAGGT
GAAGATCCTTTTTGATAATCTCATGACCAAAATCCCTTAACGTGAGTTTTCG
TTCCACTGAGCGTCAGACCCCGTAGAAAAGATCAAAGGATCTTCTTGAGAT
CCTTTTTTTCTGCGCGTAATCTGCTGCTTGCAAACAAAAAAACCACCGCTA
CCAGCGGTGGTTTGTTTGCCGGATCAAGAGCTACCAACTCTTTTTCCGAAG
GTAACTGGCTTCAGCAGAGCGCAGATACCAAATACTGTTCTTCTAGTGTAG
CCGTAGTTAGGCCACCACTTCAAGAACTCTGTAGCACCGCCTACATACCTC
GCTCTGCTAATCCTGTTACCAGTGGCTGCTGCCAGTGGCGATAAGTCGTGT
CTTACCGGGTTGGACTCAAGACGATAGTTACCGGATAAGGCGCAGCGGTCG
GGCTGAACGGGGGGTTCGTGCACACAGCCCAGCTTGGAGCGAACGACCTA
CACCGAACTGAGATACCTACAGCGTGAGCTATGAGAAAGCGCCACGCTTCC
CGAAGGGAGAAAGGCGGACAGGTATCCGGTAAGCGGCAGGGTCGGAACA
GGAGAGCGCACGAGGGAGCTTCCAGGGGGAAACGCCTGGTATCTTTATAG
TCCTGTCGGGTTTCGCCACCTCTGACTTGAGCGTCGATTTTTGTGATGCTCG
TCAGGGGGGCGGAGCCTATGGAAAAACGCCAGCAACGCGGCCTTTTTACG
GTTCCTGGCCTTTTGCTGGCCTTTTGCTCACATGTTCTTTCCTGCGTTATCCC
CTGATTCTGTGGATAACCGTATTACCGCCTTTGAGTGAGCTGATACCGCTCG
CCGCAGCCGAACGACCGAGCGCAGCGAGTCAGTGAGCGAGGAAGCGGAA
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