Supplementary Information

Isolation of a Non-genomic Origin Fluoroquinolone Responsive Regulatory Element Using a Combinatorial Bioengineering Approach.

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Supplementary Table 1.

Bacterial strains used in the study.

Genotype and description of each strain as acquired from EcoCyc (http://ecocyc.org/).

Strains	Genotype and description
E.coli DH5α	F^-, *deo*R, *end*A1, *gyr*A96, *hsd*R17 (rK^-/mK^+), *rec*A1, *pho*A, *rel*A1, *thi*-1, Δ(*lac* ZYA-*arg*F), U169ϕ80d*lac*ZΔM15λ^-, *sup*E44
E. coli MG1655	F^- λ^- ilvG- rfb-50 rph-1
E. coli RFM443	(rpsL galK2 Δlac74) streptomycin resistant
E. coli BW25113 (Wild type)	*F-, Δ(araD-araB)567, ΔlacZ4787(::rrnB-3), λ-, rph-1, Δ(rhaD-rhaB)568, hsdR514*
E. coli BW25113 Δ*cra*	*F-, Δ(araD-araB)567, ΔlacZ4787(::rrnB-3), λ-, rph-1, Δ(rhaD-rhaB)568, hsdR514,* Δ*cra* (deletion mutant of **C**atabolite **r**epressor **a**ctivator)
E. coli BW25113 Δ*cueR*	*F-, Δ(araD-araB)567, ΔlacZ4787(::rrnB-3), λ-, rph-1, Δ(rhaD-rhaB)568, hsdR514,* Δ*cueR* (deletion mutant of **Cu e**fflux **r**egulator)
E. coli BW25113 Δ*dksA*	*F-, Δ(araD-araB)567, ΔlacZ4787(::rrnB-3), λ-, rph-1, Δ(rhaD-rhaB)568, hsdR514*, Δ*dksA* (deletion mutant of
E. coli BW25113 Δ*yaiA*	*F-, Δ(araD-araB)567, ΔlacZ4787(::rrnB-3), λ-, rph-1, Δ(rhaD-rhaB)568, hsdR514,* Δ*yaiA* (deletion mutant of OxyR-dependent induction of expression by hydrogen peroxide)
E. coli BW25113 Δ*ykgD*	*F-, Δ(araD-araB)567, ΔlacZ4787(::rrnB-3), λ-, rph-1, Δ(rhaD-rhaB)568, hsdR514,* Δ*ykgD* (deletion mutant of redox-sensitive transcriptional activator)
E. coli BW25113 Δ*nhaR*	*F-, Δ(araD-araB)567, ΔlacZ4787(::rrnB-3), λ-, rph-1, Δ(rhaD-rhaB)568, hsdR514,* Δ*nhaR* (deletion mutant of "**N**a^+/**H**$^+$ **a**ntiporter **R**egulator)
E. coli BW25113 Δ*atoC*	*F-, Δ(araD-araB)567, ΔlacZ4787(::rrnB-3), λ-, rph-1, Δ(rhaD-rhaB)568, hsdR514,* Δ*atoC* (deletion mutant of antizyme protein inhibitor of

	ornithine decarboxylase)
E. coli BW25113 Δ*fadR*	*F-, Δ(araD-araB)567, ΔlacZ4787(::rrnB-3), λ-, rph-1, Δ(rhaD-rhaB)568, hsdR514,* Δ*fadR* (deletion mutant of **F**atty **a**cid **d**egradation **R**egulon)
E. coli BW25113 Δ*acr*	*F-, Δ(araD-araB)567, ΔlacZ4787(::rrnB-3), λ-, rph-1, Δ(rhaD-rhaB)568, hsdR514,* Δ*acr* (deletion mutant of acr gene involved with **Acr**idine transport)
E. coli BW25113 Δ*mprA*	*F-, Δ(araD-araB)567, ΔlacZ4787(::rrnB-3), λ-, rph-1, Δ(rhaD-rhaB)568, hsdR514,* Δ*mprA* (deletion mutant of **m**ultidrug **r**esistance **r**egulator)
E. coli BW25113 Δ*fur*	*F-, Δ(araD-araB)567, ΔlacZ4787(::rrnB-3), λ-, rph-1, Δ(rhaD-rhaB)568, hsdR514,* Δ*fur* (deletion mutant of **F**erric **U**ptake **R**egulation)
E. coli BW25113 Δ*recA*	*F-, Δ(araD-araB)567, ΔlacZ4787(::rrnB-3), λ-, rph-1, Δ(rhaD-rhaB)568, hsdR514,* Δ*recA* (deletion mutant of Recombination protein A)
E. coli BW25113 Δ*cusR*	*F-, Δ(araD-araB)567, ΔlacZ4787(::rrnB-3), λ-, rph-1, Δ(rhaD-rhaB)568, hsdR514,* Δ*cusR* (deletion mutant of **Cu**-**s**ensing **r**egulator)
E. coli BW25113 Δ*lrp*	*F-, Δ(araD-araB)567, ΔlacZ4787(::rrnB-3), λ-, rph-1, Δ(rhaD-rhaB)568, hsdR514,* Δ*lrp* (deletion mutant of **L**eucine-responsive **r**egulatory **p**rotein)
E. coli BW25113 Δfis	*F-, Δ(araD-araB)567, ΔlacZ4787(::rrnB-3), λ-, rph-1, Δ(rhaD-rhaB)568, hsdR514,* Δfis (deletion mutant of **f**actor for **i**nversion **s**timulation)
E. coli BW25113 Δ*arcA*	*F-, Δ(araD-araB)567, ΔlacZ4787(::rrnB-3), λ-, rph-1, Δ(rhaD-rhaB)568, hsdR514,* Δ*arcA* (deletion mutant of dual transcriptional regulator for **A**noxic **r**edox **c**ontrol)
E. coli BW25113 Δ*fnr*	*F-, Δ(araD-araB)567, ΔlacZ4787(::rrnB-3), λ-, rph-1, Δ(rhaD-rhaB)568, hsdR514,* Δ*fnr* (deletion mutant of primary transcriptional regulator, FNR activates genes involved in anaerobic metabolism and represses genes involved in aerobic metabolism)
E. coli BW25113 Δ*hu*	*F-, Δ(araD-araB)567, ΔlacZ4787(::rrnB-3), λ-, rph-1, Δ(rhaD-rhaB)568, hsdR514,* Δ*hu* (deletion mutant of The HU protein is a small DNA-binding protein that is considered a global regulatory protein and shares properties with histones, which play an important role in nucleoid organization and regulation).
E. coli BW25113 Δ*sodA*	*F-, Δ(araD-araB)567, ΔlacZ4787(::rrnB-3), λ-, rph-1, Δ(rhaD-rhaB)568, hsdR514,* Δ*sodA* (deletion mutant of superoxide dismutases)
E. coli BW25113 Δ*stpA*	*F-, Δ(araD-araB)567, ΔlacZ4787(::rrnB-3), λ-, rph-1, Δ(rhaD-rhaB)568, hsdR514,* Δ*stpA* (deletion mutant of StpA protein **S**uppressor of ***t****d* **p**henotype **A**," is a nucleoid-associated multifunctional protein that acts as a transcriptional repressor, in chromosomal DNA packaging, and as a chaperone
E. coli BW25113 Δ*oxyR*	*F-, Δ(araD-araB)567, ΔlacZ4787(::rrnB-3), λ-, rph-1, Δ(rhaD-rhaB)568, hsdR514,* Δ*oxyR* (deletion mutant of oxidative stress regulator)
E. coli BW25113 Δ*hns*	*F-, Δ(araD-araB)567, ΔlacZ4787(::rrnB-3), λ-, rph-1, Δ(rhaD-rhaB)568,*

	hsdR514, Δ*hns* (deletion mutant of **H**istone-like **n**ucleoid **s**tructuring protein)
E. coli BW25113 Δ*crp*	*F-, Δ(araD-araB)567, ΔlacZ4787(::rrnB-3), λ⁻, rph-1, Δ(rhaD-rhaB)568, hsdR514,* Δ*crp* (deletion mutant of **c**AMP **r**eceptor **p**rotein)
E. coli BW25113 Δ*marA*	*F-, Δ(araD-araB)567, ΔlacZ4787(::rrnB-3), λ⁻, rph-1, Δ(rhaD-rhaB)568, hsdR514,* Δ*marA* (deletion mutant of **m**ultiple **a**ntibiotic **r**esistance regulatory protein)
E. coli BW25113 Δ*baeR*	*F-, Δ(araD-araB)567, ΔlacZ4787(::rrnB-3), λ⁻, rph-1, Δ(rhaD-rhaB)568, hsdR514,* Δ*baeR* (deletion mutant of **b**acterial **a**daptive r**e**sponse)
E. coli BW25113 Δ*cpxR*	*F-, Δ(araD-araB)567, ΔlacZ4787(::rrnB-3), λ⁻, rph-1, Δ(rhaD-rhaB)568, hsdR514,* Δ*cpxR* (deletion mutant of **c**onjugative **p**lasmid gene e**x**pression regulatory protein)

Supplementary Table 2.

Plasmids used in the study

Plasmid Name	**Description/Resistance**	**References**
pNYL-MCS11	Plasmid derived from pZE21	2, 3
pNYL-GFP	GFP gene was amplified from pPROBE-TT'-GFP and was cloned in pNYL-MCS11 plasmid vector on BamHI restriction site.	This study
pNYL-ibsC	Genetic locus coding for toxic peptide ibsC was amplified from *E. coli* genome and cloned in pNYL-MCS11 vector at BamHI restriction site	This study
pNal::ibsC	Reporter plasmid containing FQ responsive regulating element fused with gene encoding toxic peptide IbsC. Vector backbone codes for kanamycin resistance.	This study
p*Nal::GFP*	Reporter plasmid containing FQ responsive promoter fused with GFP.	This study
pRx::ibsC	Control plasmid containing pNYL-MCS backbone without FQ responsive promoter sequences.	This study
pNal::ibsC-amp	From vector backbone of *pNal::ibsC,* kanamycin resistant selection marker gene was replaced by ampicillin resistant gene	This study
pRx::ibsC-amp	From vector backbone of *pRx::ibsC,* kanamycin resistant selection marker gene was replaced by ampicillin resistant gene.	This study

Supplementary Figure S1.

Alignment of promoter regions of representative prokaryotic stress responsive genes using ClustalW2 online bioinformatics program.

Supplementary Figure S2. Schematic representation of cloning and screening of combinatorial oligonucleotide library.

A) Commercially synthesized single stranded combinatorial oligonucleotide library were converted in to double stranded DNA fragment and double digested with *XhoI* and *Hind*III. Double digested library were ligated with *Xho*I-*Hind*III digested fragment of pNYL-ibsC/GFP plasmid vector and transformed in *E. coli* RFM443 (Details are available in material and method section). B) To screen the inducible regulatory elements, a live and dead screening strategy has been adopted. Bacterial colonies were selected from the transformed plate and inoculated individually into 96 well plate containing 200 µl LB broth. The grown cultures were then replica plated on a 96 well plate which contained various toxicants including DNA damaging [fluoroquinolones, mitomycin C], protein damaging [ethanol] and heavy metals [sodium arsenite, cadmium chloride, lead chloride] at sub-lethal concentrations and absorbance after incubation was measured at 600 nm. C) The presence of toxicants induces the expression of the toxic peptide IbsC which leads to the killing of the bacterial cells. The IbsC toxic peptide based reporter system selectively screens out the clones harboring functional combinatorial promoters against leaky and constitutive promoters.

Supplementary Figure S3.

E. coli BL21 DE3 strain harboring pET28a-hns was induced with 1mM IPTG Analysis of the purified H-NS protein on 12.5% sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE).

Supplementary Figure S4.

Nucleotide sequence of clones representing constitutive promoters (a,b,c,d,e,f,g and h) covering a wide range of GFP fluorescence intensity.

Supplementary Figure S5.

Multiple sequence alignment of FQ-responsive regulatory element with the selected stress promoters from *E. coli* genome. FQ-responsive regulatory element displayed no homology with stress promoter sequences encoded in *E. coli* genome.

Supplementary Figure S6.

Response of FQ responsive promoter in bacterial host lacking efflux pump *tolC* gene and wild type *E. coli* strain in the presence of nalidixic acid. A-B) Wild type Δ*tolC* bacterial strains harboring *pNal::ibsC-amp* (test) and *pRx::ibsC-amp* (control) plasmid was induced with nalidixic acid.

Supplementary Figure S7.

Growth pattern of various stress regulator gene knock out strains of *E. coli* harboring p*Nal::ibsC-amp* and p*Rx::ibsC-amp* fusion system. The growth assays were carried out in duplicate for the *E. coli* mutant strain harboring p*Nal::ibsC-amp* plasmid (test) and p*Rx::ibsC-amp* plasmid (control).

Supplementary Figure S8. *In-silico* DNA curvature analysis of FQ responsive (Nal) promoter and their mutant variants at spacer region ('all A' and 'all T').

Figure S1

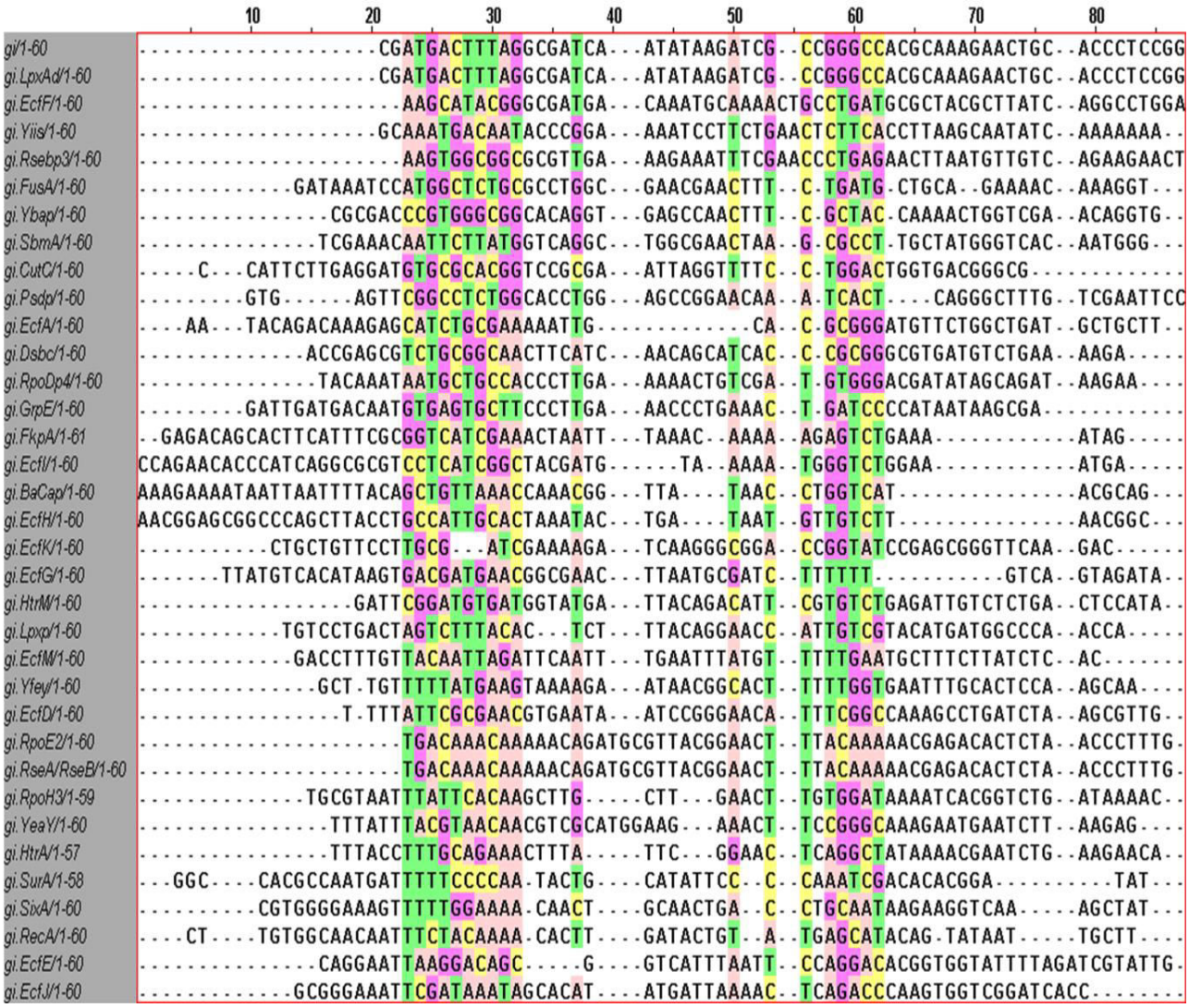
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Figure S2

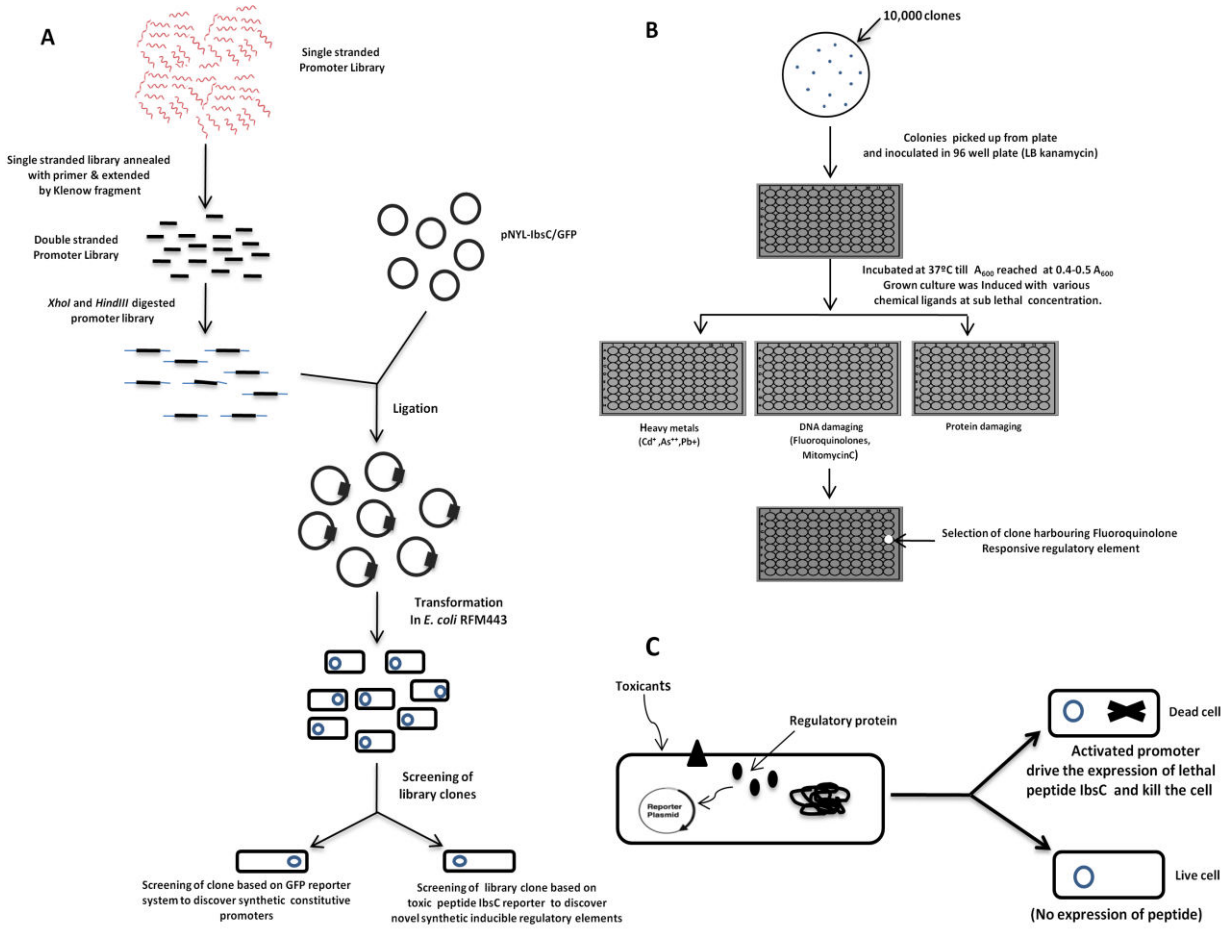
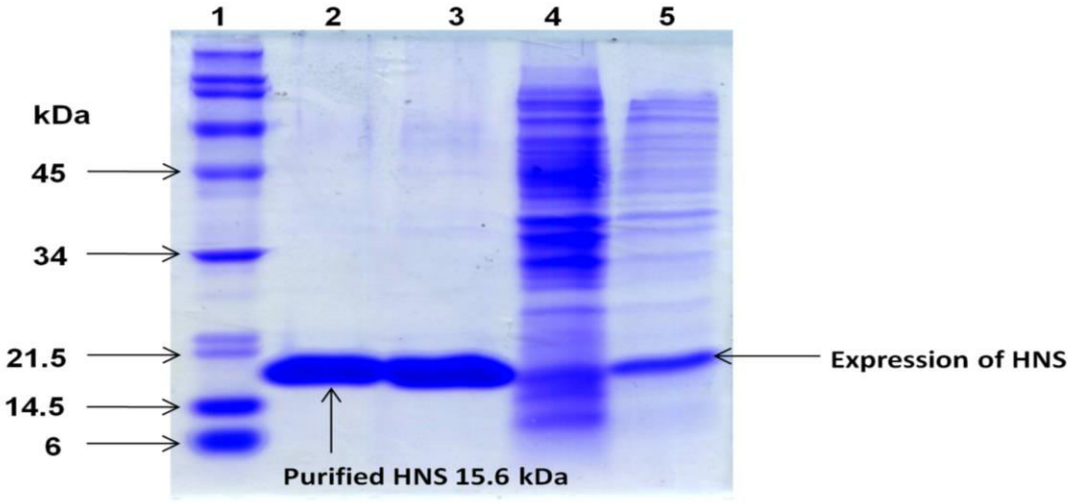

A
Single stranded Promoter Library
Single stranded library annealed with primer & extended by Klenow fragment
Double stranded Promoter Library
XhoI and HindIII digested promoter library
pNYL-IbsC/GFP
Ligation
Transformation In E. coli RFM443
Screening of library clones
Screening of clone based on GFP reporter system to discover synthetic constitutive promoters
Screening of library clone based on toxic peptide IbsC reporter to discover novel synthetic inducible regulatory elements
B
10,000 clones
Colonies picked up from plate and inoculated in 96 well plate (LB kanamycin)
Incubated at 37ºC till A600 reached at 0.4-0.5 A600
Grown culture was Induced with various chemical ligands at sub lethal concentration.
Heavy metals (Cd+,As++,Pb+)
DNA damaging (Fluoroquinolones, MitomycinC)
Protein damaging
Selection of clone harbouring Fluoroquinolone Responsive regulatory element
C
Toxicants
Regulatory protein
Reporter Plasmid
Dead cell
Activated promoter drive the expression of lethal peptide IbsC and kill the cell
Live cell
(No expression of peptide)

Figure S3



Lane-1 Broad range protein marker

Lane-2 Purified fraction1

Lane-3 Purified fraction2

Lane-4 Uninduced

Lane-5 *pET28a*-hns cellular extract induced with 0.5 mM IPTG

Figure S4

Clone-a

CTTTTGTTTACGTCTTCCCTCGAGATCAGTCAAATACAGCGCAACTCGTAAAAAGGGGGAGTTTCCAACAATCCAGGCAGTCATAT
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CTGGTCGCACCAACCGGCGAGTCATATCTAAGCTTGATATCGAATTCCTGCAGCCCGGGGGATCCATAAGGAGGAAAAACATATG
AGTAAAGGAGAAGAACTTTTCACTGGAGT

Clone -b

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Clone- c

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Clone-d

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Clone f

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Clone g

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Clone-h

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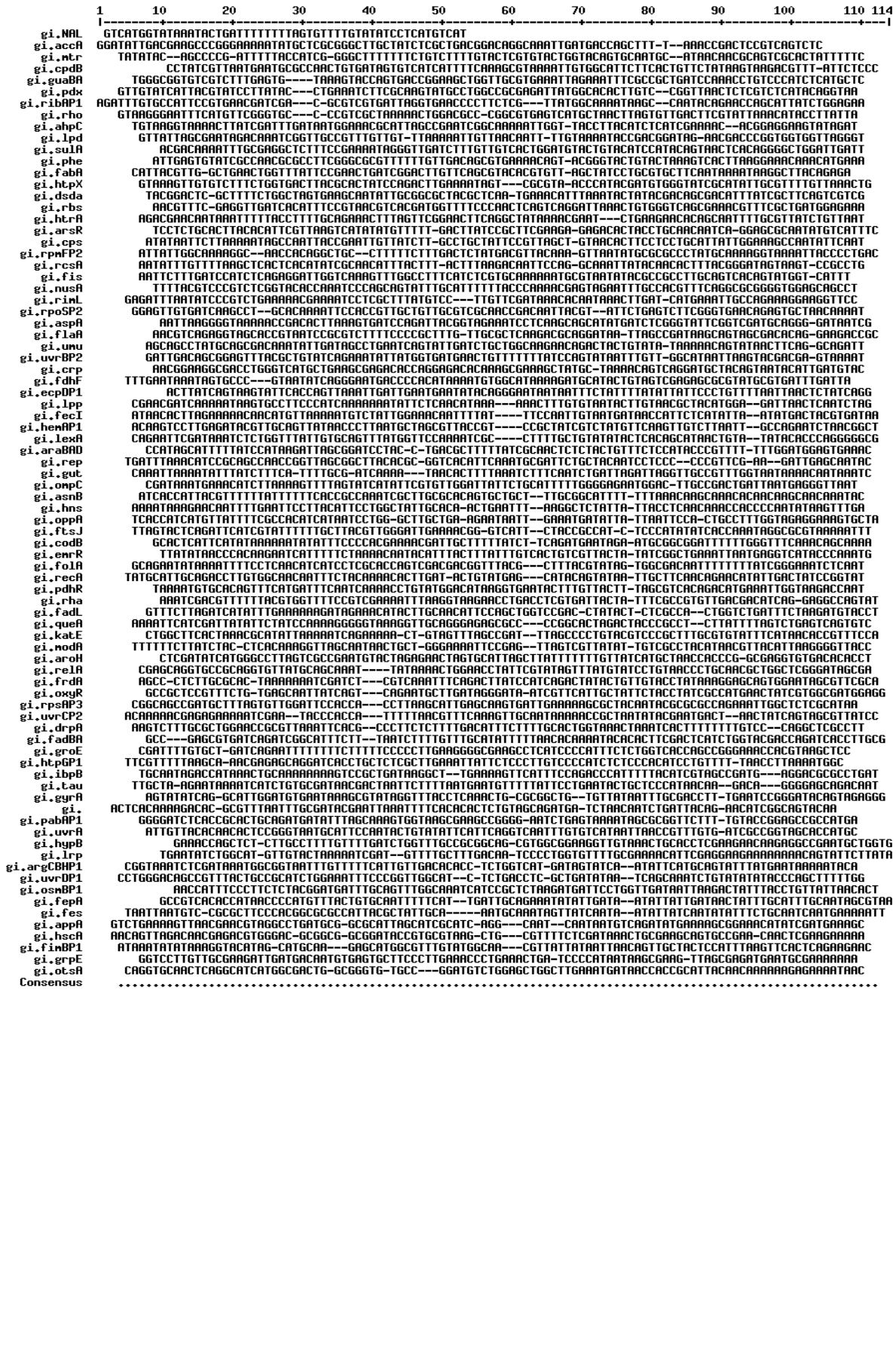
Figure S5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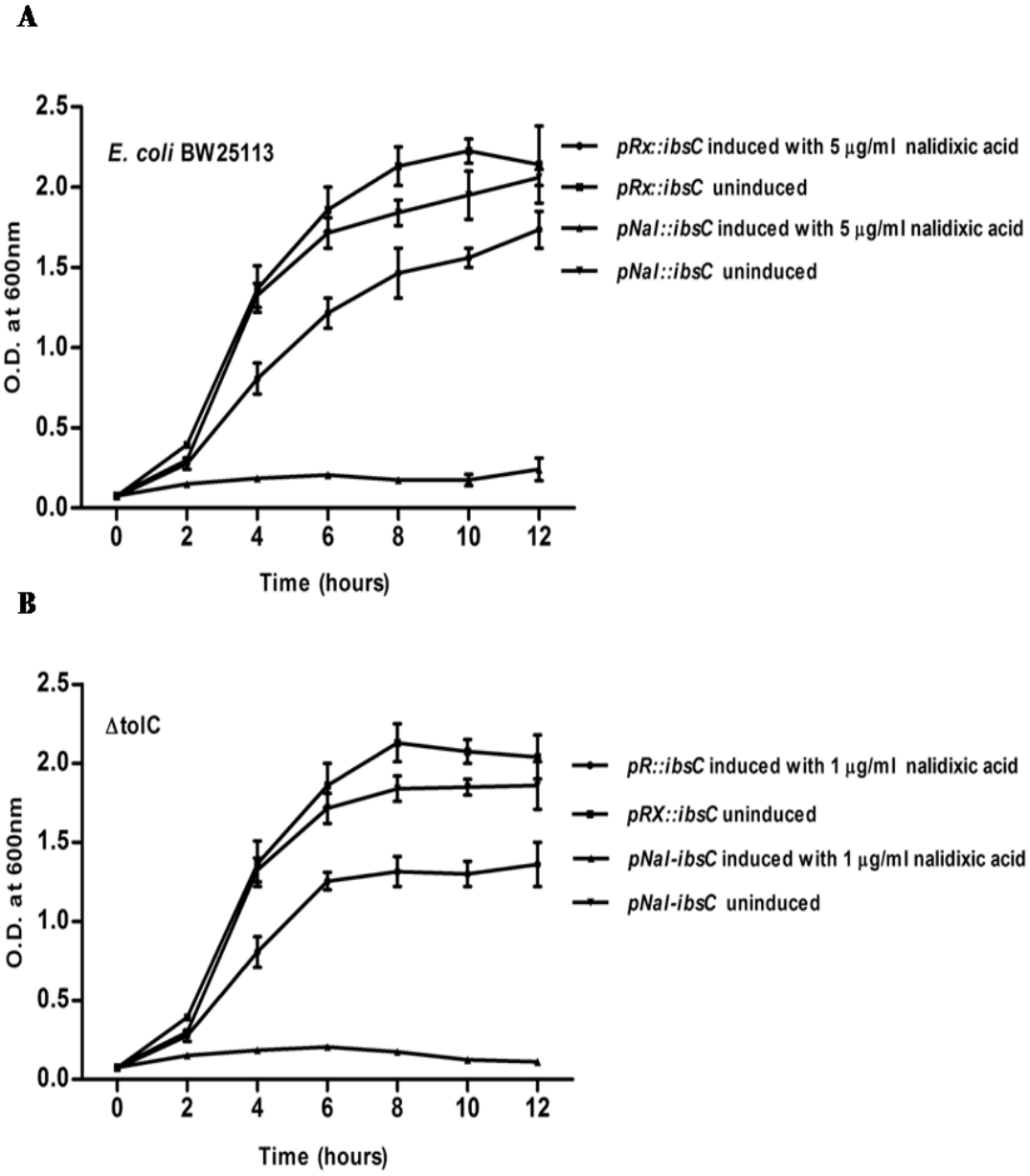
gi.otsA
Consensus

Figure S6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E. coli BW25113
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Time (hours)
pRx::ibsC induced with 5 μg/ml nalidixic acid
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pNal::ibsC induced with 5 μg/ml nalidixic acid
pNal::ibsC uninduced
B
ΔtolC
O.D. at 600nm
Time (hours)
pR::ibsC induced with 1 μg/ml nalidixic acid
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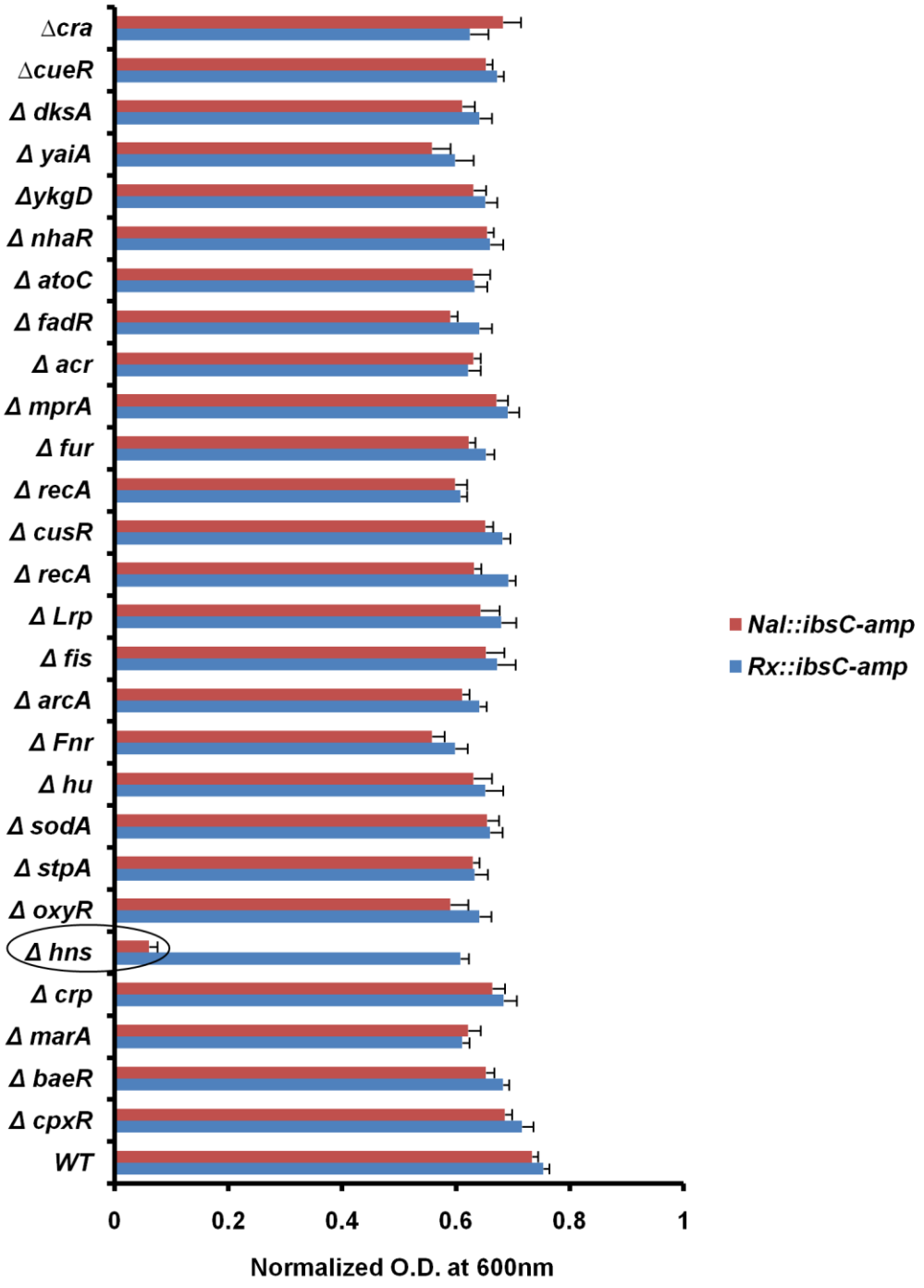
pNal-ibsC uninduced

Figure S7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Δ yaiA
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Δ nhaR
Δ atoC
Δ fadR
Δ acr
Δ mprA
Δ fur
Δ recA
Δ cusR
Δ recA
Δ Lrp
Δ fis
Δ arcA
Δ Fnr
Δ hu
Δ sodA
Δ stpA
Δ oxyR
Δ hns
Δ crp
Δ marA
Δ baeR
Δ cpxR
WT
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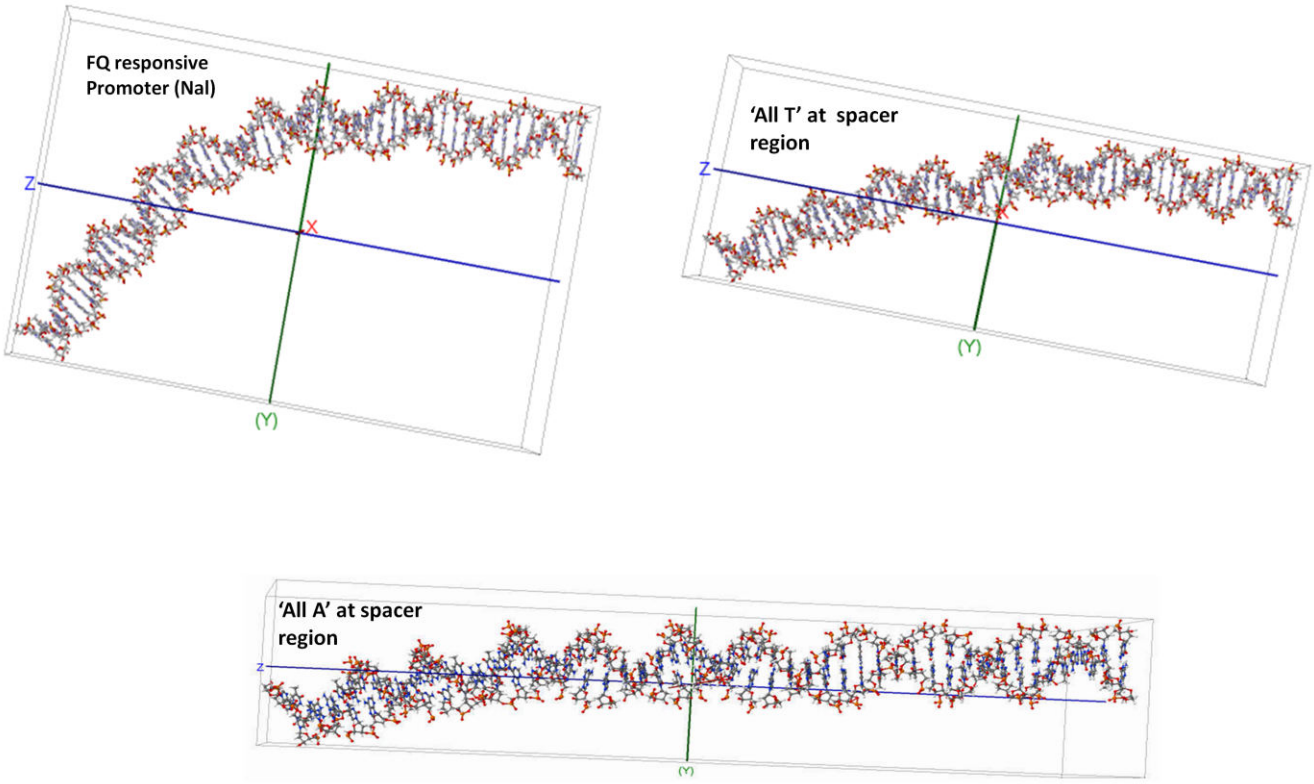
Nal::ibsC-amp
Rx::ibsC-amp

Figure S8


FQ responsive
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(Y)
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region
Z
X
(Y)
'All A' at spacer
region
z
(Y)