

Table S1. Strains and plasmids. The genotypes of all strains of *E. coli* utilized or constructed in this study and information about the plasmids used in this study.

Bacterial strains and plasmids	Genotype or relevant characteristics	Source or Reference
<i>E. coli</i> DH5α	Plasmid propagation strain	Invitrogen
<i>E. coli</i> S17-λpir	RK2 tra regulon, pir, host for pir-dependent plasmids	[1]
<i>E. coli</i> fnr-771(del)::kan	F-, Δ(<i>araD-araB</i>)567, Δ <i>lacZ</i> 4787(:rrnB-3), λ-, Δ <i>fnr</i> -771::kan,rph-1, Δ(<i>rhaD-rhaB</i>)568, <i>hsdR514</i>	[2]
UPEC CFT073	Blood isolate from a patient with acute pyelonephritis	[3]
LMP10	CFT073 Δ <i>lacZYA</i>	[4]
LMP11	CFT073 Δ <i>lacZYA</i> ::Chlr	[4]
	CFT073 Δ <i>fnr</i>	This study
	CFT073 Δ <i>fnr</i> ::kan	This study
CFT073 ^{OFF}	IRL, fim invertible element locked off	[5]
CFT073 ^{ON}	IRL, fim invertible element locked on	[5]
	CFT073 Δ <i>lacZYA</i> Δ <i>fnr</i>	This study
	CFT073 Δ <i>lacZYA</i> <i>fimA-lacZ</i>	This study
	CFT073 Δ <i>lacZYA</i> Δ <i>fnr</i> <i>fimA-lacZ</i>	This study
	CFT073 Δ <i>lacZYA</i> <i>fimB-lacZ</i>	This study
	CFT073 Δ <i>lacZYA</i> Δ <i>fnr</i> <i>fimB-lacZ</i>	This study
	CFT073 Δ <i>lacZYA</i> <i>fimE-lacZ</i>	This study
	CFT073 Δ <i>lacZYA</i> Δ <i>fnr</i> <i>fimE-lacZ</i>	This study
	CFT073 Δ <i>lacZYA</i> <i>papA-lacZ</i>	This study
	CFT073 Δ <i>lacZYA</i> Δ <i>fnr</i> <i>papA-lacZ</i>	This study
	CFT073 Δ <i>lacZYA</i> <i>papB-lacZ</i>	This study
	CFT073 Δ <i>lacZYA</i> Δ <i>fnr</i> <i>papB-lacZ</i>	This study
	CFT073 Δ <i>lacZYA</i> c5038- <i>lacZ</i>	[4]
	CFT073 Δ <i>lacZYA</i> Δ <i>fnr</i> c5038- <i>lacZ</i>	This study
	CFT073 Δ <i>lacZYA</i> <i>kguS-lacZ</i>	[4]
	CFT073 Δ <i>lacZYA</i> Δ <i>fnr</i> <i>kguS-lacZ</i>	This study
	CFT073 Δ <i>lacZYA</i> <i>kguR-lacZ</i>	[4]
	CFT073 Δ <i>lacZYA</i> Δ <i>fnr</i> <i>kguR-lacZ</i>	This study
	CFT073 Δ <i>lacZYA</i> <i>hlyA-lacZ</i>	This study
	CFT073 Δ <i>lacZYA</i> Δ <i>fnr</i> <i>hlyA-lacZ</i>	This study
	CFT073 Δ <i>lacZYA</i> c3565- <i>lacZ</i>	This study
	CFT073 Δ <i>lacZYA</i> Δ <i>fnr</i> c3565- <i>lacZ</i>	This study
	CFT073 Δ <i>lacZYA</i> <i>fliA-lacZ</i>	This study
	CFT073 Δ <i>lacZYA</i> Δ <i>fnr</i> <i>fliA-lacZ</i>	This study
	CFT073 Δ <i>lacZYA</i> <i>fliC-lacZ</i>	This study
	CFT073 Δ <i>lacZYA</i> Δ <i>fnr</i> <i>fliC-lacZ</i>	This study
	CFT073 Δ <i>lacZYA</i> <i>flhDC-lacZ</i>	This study
	CFT073 Δ <i>lacZYA</i> Δ <i>fnr</i> <i>flhDC-lacZ</i>	This study

Plasmids		
pET28a-(fnrD154A)2	expression plasmid of FNR protein suicide plasmid for chromosomal lacZ	[6]
pVIK112	transcriptional fusion	[7]
pGEN-MCS	low copy plasmid for complementation pGEN-MCS carrying fnr coding region and	[8]
pGEM-FNR	500bp upstream promoter region	This study
pKD3	template for λ-Red Chlr cassette	[9]
pKD4	template for λ-Red Kanr cassette	[9]
pCP20	encodes FLP recombinase for removal of resistance cassette	[9]
pKD46	λ-Red recombinase expression	[9]

1. Simon R, Priefer U, Puhler A (1983) A Broad Host Range Mobilization System for In Vivo Genetic Engineering: Transposon Mutagenesis in Gram Negative Bacteria. *Nat Biotech* 1: 784-791.
2. Baba T, Ara T, Hasegawa M, Takai Y, Okumura Y, et al. (2006) Construction of *Escherichia coli* K-12 in-frame, single-gene knockout mutants: the Keio collection. *Molecular Systems Biology* 2.
3. Welch RA, Burland V, Plunkett G, 3rd, Redford P, Roesch P, et al. (2002) Extensive mosaic structure revealed by the complete genome sequence of uropathogenic *Escherichia coli*. *Proc Natl Acad Sci U S A* 99: 17020-17024.
4. Cai W, Wannemuehler Y, Dell'Anna G, Nicholson B, Barbieri NL, et al. (2013) A novel two-component signaling system facilitates uropathogenic *Escherichia coli*'s ability to exploit abundant host metabolites. *Plos Pathogens* 9: e1003428.
5. Gunther NW, Snyder JA, Lockatell V, Blomfield I, Johnson DE, et al. (2002) Assessment of virulence of uropathogenic *Escherichia coli* type 1 fimbrial mutants in which the invertible element is phase-locked on or off. *Infection and Immunity* 70: 3344-3354.
6. Shan Y, Pan Q, Liu J, Huang F, Sun H, et al. (2012) Covalently linking the *Escherichia coli* global anaerobic regulator FNR in tandem allows it to function as an oxygen stable dimer. *Biochemical and Biophysical Research Communications* 419: 43-48.
7. Kalogeraki VS, Winans SC (1997) Suicide plasmids containing promoterless reporter genes can simultaneously disrupt and create fusions to target genes of diverse bacteria. *Gene* 188: 69-75.
8. Lane MC, Alteri CJ, Smith SN, Mobley HLT (2007) Expression of flagella is coincident with uropathogenic *Escherichia coli* ascension to the upper urinary tract. *Proceedings of the National Academy of Sciences of the United States of America* 104: 16669-16674.
9. Datsenko KA, Wanner BL (2000) One-step inactivation of chromosomal genes in *Escherichia coli* K-12 using PCR products. *Proceedings of the National Academy of Sciences of the United States of America* 97: 6640-6645.

Table S2. Oligonucleotides. Oligonucleotide sequences used as PCR primers.

Primers	Sequence (5'-3')
<i>General PCR for cloning</i>	
pGEN-FNR-F	GTACC <u>ATGGATCGAATCCCATCAGCATC</u>
pGEN-FNR-R	CCGAG <u>TCGACAGGATCGATAACAACGAGCA</u>
<i>For lacZ fusion</i>	
fimA-lacZ-5'-F	CACGG <u>AATT</u> GTTGATGCAGGCTCTGTTGA
fimA-lacZ-5'-R	GCAGT <u>CTAGA</u> TTATTGATACTGAACCTTGA
fimB-lacZ-5'-F	GTCGG <u>AATT</u> CCCGATTGAGGATTTCGGATA
fimB-lacZ-5'-R	CCAGT <u>CTAGACT</u> ATAAAACAGCGTGACGCT
fimE-lacZ-5'-F	GACGG <u>AATT</u> CGGCATGGATGCGTATTAGT
fimE-lacZ-5'-R	CGAGT <u>CTAGAT</u> CAAACTTCTTCTTTTA
papA-lacZ-5'-F	CACGG <u>AATT</u> CATCAGTCGGTCAGGAAATGC
papA-lacZ-5'-R	GCAGT <u>CTAGAG</u> AGCAGCATATGCACCAAAA
papB-lacZ-5'-F	CACGG <u>AATT</u> GGCCCCTGGATATATGCTTC
papB-lacZ-5'-R	GCAGT <u>CTAGA</u> CTCCATCATGCCCTGTTCAGA
c5038-lacZ-5'-F	AGTC <u>GAATT</u> CTGGTGGTAATGCGGAAGAAC
c5038-lacZ-5'-R	ATCGT <u>CTAGAT</u> ATGCCCAAGTGGCAGAAGG
kguS-lacZ-5'-F	ATGCG <u>AATT</u> CTCGCTTCTGGCGAGAAGG
kguS-lacZ-5'-R	GCTGT <u>CTAGAG</u> AAAACCGCGAGCATGATAAG
kguR-lacZ-3'-F	ATCG <u>GAATT</u> CTGTTATTGCAGCGACCAAGG
kguR-lacZ-3'-R	GCACT <u>CTAGATT</u> AGCTGGATGATTCTGGTC
hlyA-lacZ-5'-F	ATCG <u>GAATT</u> CTATTGATTCCGGGAT
hlyA-lacZ-5'-R	ACCGT <u>CTAGATT</u> ATGCTGAGCTGTC
c3565-lacZ-5'-F	GCTGT <u>CTAGAG</u> AAAACCGCGAGCATGATAAG

c3565-lacZ-5'-R	ATCG <u>GAATTCTGTTATTGCAGCGACCAAGG</u>
hlyD-lacZ-5'-F	CAC <u>GGAAATTC</u> TTCGGGAAAAGTTCAGCAAC
hlyD-lacZ-5'-R	GCAG <u>TCTAGA</u> TTAACGCTCATGTAAACTTCT
fliA-lacZ-3'-F	CACG <u>CCCGGG</u> ATCAGGCCTACAAGGGGAAT
fliA-lacZ-3'-R	GCAG <u>TCTAGA</u> GCGTTCGACGGCATTAAAGTA
fliC-lacZ-3'-F	CAC <u>GGAAATTC</u> CGACACGTAAAACGAATACCG
fliC-lacZ-3'-R	GCAG <u>TCTAGA</u> CGCAGACTGGTTCTTGTGA
flhDC-lacZ-3'-F	CAC <u>GGAAATTC</u> CGGTGAGACCGCATAAAAAT
flhDC-lacZ-3'-R	GCAG <u>TCTAGA</u> CCCAGGTCATAAACCAGTCG

For Deletion^a

Del-fnr-F	GACGGTTATGCCAGACCACT
Del-fnr-R	AAGCGACAAGCTTCGTGAAT

For ON/OFF switch

fimEin F	GGCATGCTTGTGGTTATGAA
fimAin R	TTTCATGCTGCTTCCTTT

For EMSA

Inside negative control-For1	ATCTGTGTGGTAAGAGAAC
Inside negative control-Rev1	TGGTGCGCCATGGGATATTG
PromfimI-For	TTTGCAGAGCCAGTACGTTG
PromfimI-Rev	GTTGATGCAGGCTCTGTTGA
PrompapI-For	TTCACCCGTTTCAGAAGC
PrompapI-Rev	AAAATCCGCACACTGACCAT
PromkguR-For	AAGCCATAACGTTCCGCTTC
PromkguR-Rev	TTGCTACTGTTGCCGCTAC

Prom3565-For	CAGCGTAACCACAGAGGATG
Prom3565-Rev	CGCAACAGAGCTGCAATATC
PromfliC-For	CGCAGACTGGTTCTTGTGA
PromfliC-Rev	GGGAATAAGGGGCAGAGAAA
PromydfZ-For	GCGACTGGTTAGCGAAGAG
PromydfZ-Rev	TGGTGATTGCGTTACGGTTA

-
- Underlined are restriction cutting sites;
 - Capital letters represent homologous fragments of the deleted genes.