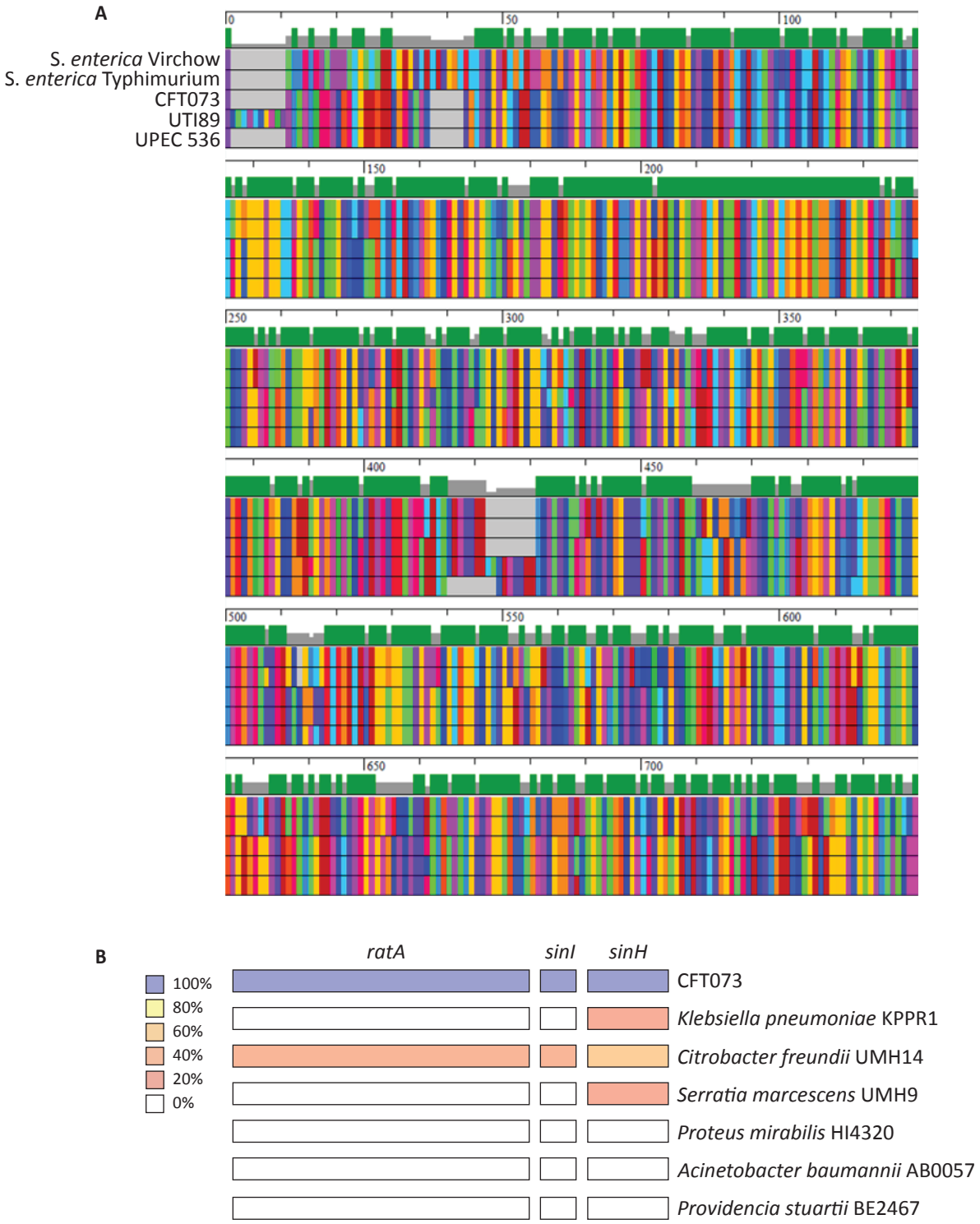
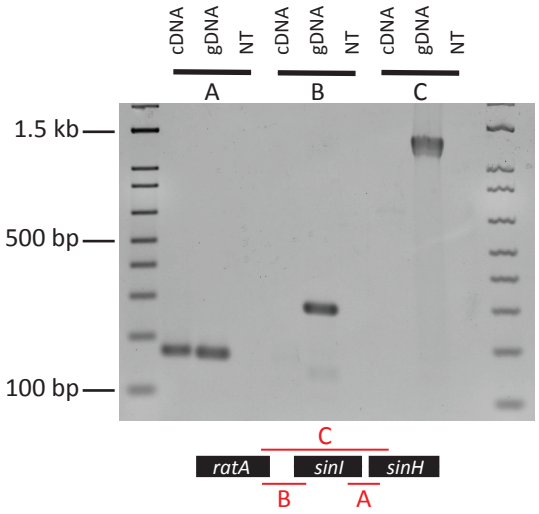
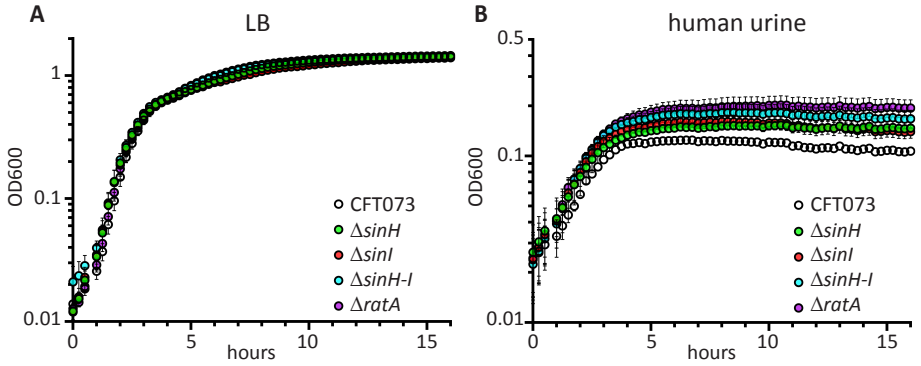


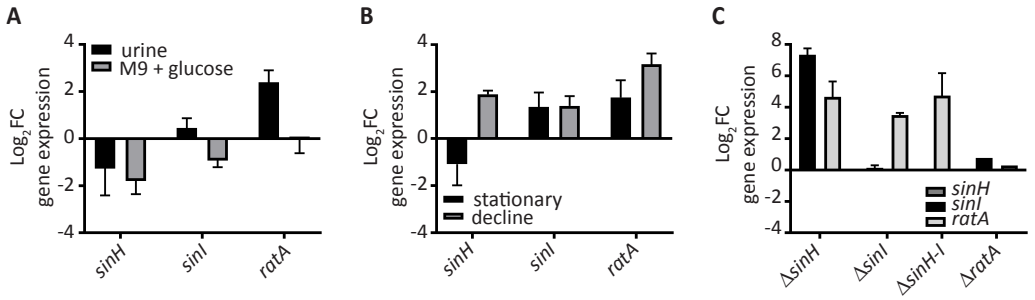
SUPPLEMENTAL FIG 1. The prevalence of intimin-like adhesins in other uropathogens. (A) The amino acid sequence for the CFT073 SinH-like protein is compared to both *Salmonella* species and UPEC in PATRIC. Consensus across all strains is marked in green and differences indicated in gray running along the top of the figure. Each amino acid is displayed in a unique color for visual display of protein alignment. (B) PATRIC protein blast was used to compare the SinH-RatA locus in CFT073 across multiple other uropathogen species. The colors indicate the percent amino acid homology in each listed strain, ranging from 0-100% identity.



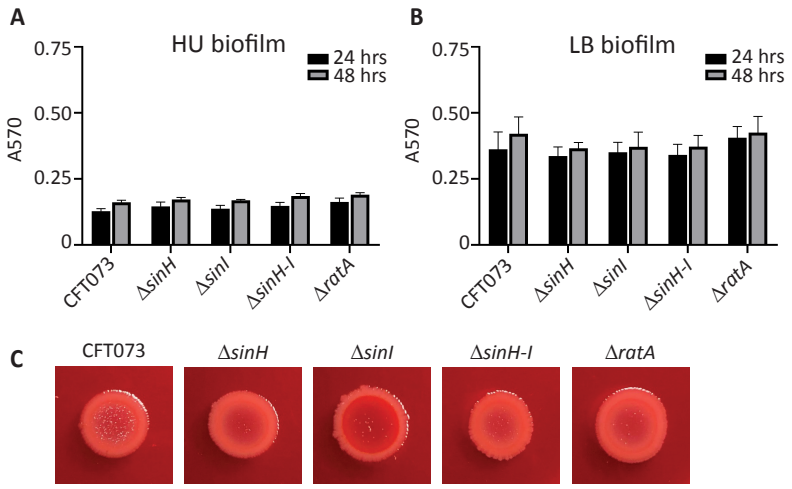
SUPPLEMENTAL FIG 2. *sinH* and *sinI* are co-transcribed, but *ratA* is not. PCR was performed on CFT073 cDNA to determine if putative intimin-like genes are co-transcribed as an operon. Primers were designed to span the junction of *sinH* and *sinI*, *sinI* and *ratA*, and *sinH* to *ratA*. CFT073 gDNA was used as a positive control for each primer pair and no template (NT) served as a negative control.



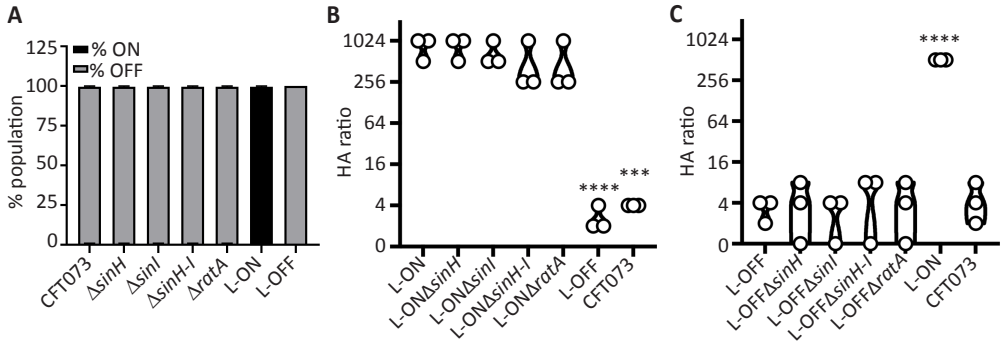
SUPPLEMENTAL FIG 3. Intimin-like mutants do not display *in vitro* growth defects. Bacterial growth of WT CFT073 and mutants Δ*sinH*, Δ*sinI*, Δ*sinH-sinI* and Δ*ratA* was measured for 16 h in (A) LB medium and (B) filter-sterilized pooled human urine. Average OD_{600} for each strain was plotted at every 15 min. Data shown are the average of biological triplicates (n=3), error bars indicate the standard error of the mean and are sometimes not visible due to symbol size.



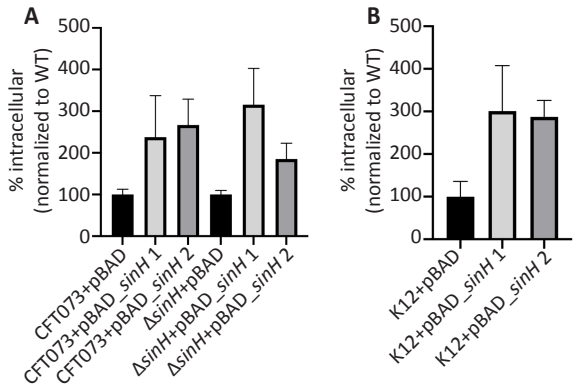
SUPPLEMENTAL FIG 4. Coordinated upregulation of the intimin-like locus in static and late-stationary phase growth conditions. WT CFT073 was cultured in specific in vitro conditions to isolate RNA for resulting qPCR to determine expression levels of *sinH*, *sinI*, and *ratA*. The comparative threshold cycle (CT) method was used to determine the relative $\log_2$ fold-change of each strain in specified conditions. (A) Growth of CFT073 in human urine (black bars) or M9 + 0.4% dextrose (gray bars) was normalized to LB. Growth was conducted to mid-log phase in shaking conditions at 37°C. Bars indicate the mean of five biological replicates, error bars indicate SEM. (B) Growth in either stationary conditions (black bars) or 48 h cultures cultured at 37°C shaking overnight and then another overnight incubation on the benchtop (gray bars) was normalized to mid-log cultures in shaking conditions. Bars indicate the mean of five biological replicates, error bars indicate SEM. (C) Mutant strains were cultured in the 48 h culture model described previously, and normalized to WT CFT073 in shaking conditions. The expression of *sinH* (dark gray), *sinI* (black), and *ratA* (light gray) are indicated in each strain, bar height represent means and error bars are SEM.



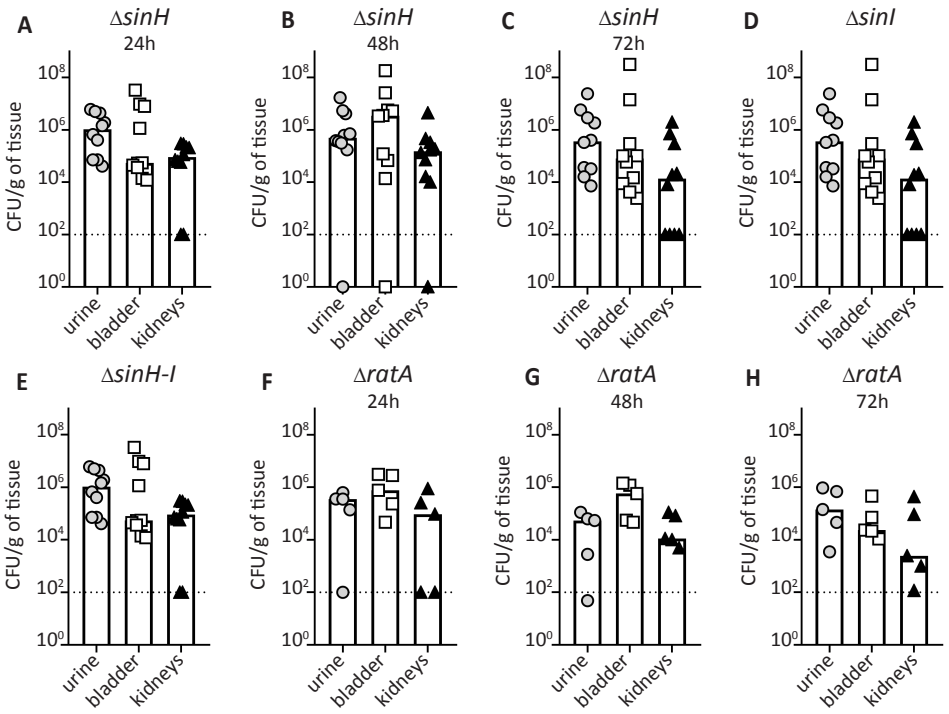
SUPPLEMENTAL FIG 5. The role of intimin-like adhesins in biofilm formation. The average absorbance readings measured at 570 nm are displayed. (A and B) Both 24 h (black) and 48 h (gray) biofilms were measured after incubation at 30°C. Bar height indicates the mean of five independent biological trials with standard errors of the mean represented by error bars. For biofilm assays in both (A) LB medium (B) and human urine, WT CFT073 is compared to mutants Δ*sinH*, Δ*sinI*, Δ*sinH-sinI* and Δ *ratA*. (C) Bacteria cultured for 48 h were spotted onto congo red agar plates and incubated at 30°C for 72h before imaging. The image shown is a representation of multiple independent trials. Lighter colors on congo red agar indicate a lack of curli pili, while dark red indicates the presence of curli. Moderate differences can be seen comparing WT CFT073 to intimin-like mutants.



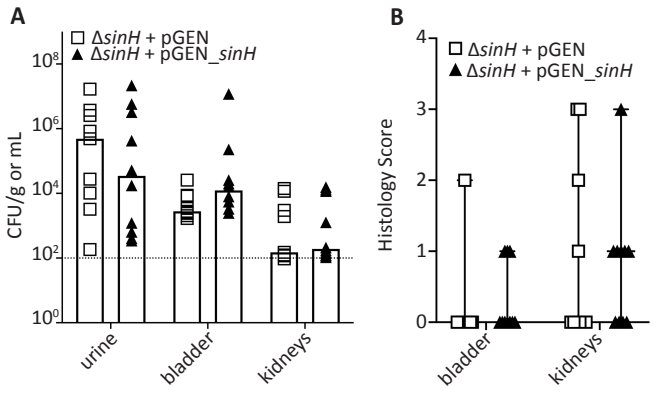
SUPPLEMENTAL FIG 6. Type 1 fimbriae influence on *ila* mutants. (A) The invertible element assay was performed on WT, *ila* mutants, CFT Fim L-ON, and Fim L-OFF were cultured shaking overnight at 37°C, and then statically on the benchtop for 24h. Image J software was used to quantify the intensity of Fim-ON and Fim-OFF sized bands. The mean of three independent trials of both the percent on (black) and off (gray) of the total population are displayed with SEM. (B and C) Bacterial strains were cultured for 72 h statically at 37°C. 1:2 serial dilutions of culture were co-incubated with erythrocytes, mixed, and observed after 1 h. The last titer detected with positive hemagglutination is recorded for each strain, and each biological replicate is indicated by a point (n=3). Violin plot width indicates the distribution of data. If significant, P-values determined by one-way ANOVA with Dunnett's multiple comparisons test, compared to L-ON or L-OFF parental strain, are indicated (***, P<0.001; ****, P<0.0001).



SUPPLEMENTAL FIG 7. SinH alone can increase intracellular invasion into bladder cells. (A and B) UPEC cell internalization was determined in T24 bladder cells. After 1 h co-culture of bacteria with host cells (+0.4% arabinose), the media was supplemented with gentamicin to kill extracellular bacteria. Intracellular bacteria were enumerated and normalized to the input. Data shown are normalized to each parental strain containing the empty vector plasmid. Bars indicate the mean of internalized UPEC CFU burden from three biological replicates, with SEM shown.



SUPPLEMENTAL FIG 8. CFU output from murine ascending UTI. Total CFU output per gram of tissue (or mL of urine) is plotted for each individual mouse in the (A-C) Δ*sinH*, (D) Δ*sinI*, (E) Δ*sinH-I*, and (F-H) Δ*ratA* co-challenges against WT CFT073 with the bar indicating the median. Each point represents a mouse shown in Figure 6. The dashed line denotes the limit of detection.



SUPPLEMENTAL FIG 9. Partial complementation of Δ*sinH* in vivo. (A) Ten mice were transurethrally inoculated with either Δ*sinH*+pGEN or Δ*sinH*+pGEN_*sinH* for 48 h. Total CFU output per gram of tissue (or mL of urine) is plotted for each individual with the bar indicating the median bacterial burden. The dashed line indicates the limit of detection. (B) Corresponding histopathology inflammation scores were determined from bladders and kidneys of individually challenged mice (n=10). Each dot represents a single organ and the line indicates median with range.

Table S1. Strains and plasmids used in this study

Strains	**Antibiotic Resistance**	**Reference**
CFT073		(1)
CFT073Δ*sinH*	Kan^R	This study
CFT073Δ*sinI*	Kan^R	This study
CFT073Δ*sinH-sinI*	Kan^R	This study
CFT073Δ*ratA*	Kan^R	This study
CFT073 ΔIRL, *fim* invertible element locked on		**(2)**
CFT073 ΔIRL, *fim* invertible element locked off		**(2)**
Plasmids		
pGEN-MCS	Amp^R	(3)
pBAD	Amp^R	(4)

Table S2. Primer sequences used in this study.	
	5′- 3′ sequence
Primer Name	Primers used for co-transcription determination
sinH start	CAGGGAAAGTGCATGCTGCG
sinH end	AACCTATGGCGCACAAGCGGG
sinI start	TAATGCGATCCGGGTCAGACC
sinI end	AAGAGGCACTTTCCATCCCG
ratA start	TGCAGATTCTGCCCATGACC
	Primers used for qPCR
sinH qPCR F	TGGACATCGCCGTTTAGGTTTAG
sinH qPCR R	TGGCACTGGCAGGTTATTGT
sinI qPCR F	TTCCGAATAGAGCCACGGAATG
sinI qPCR R	GTAACCAGACTTCGGCACGAATA
ratA qPCR F	TACCGCGTTCACCATCACTAAA
ratA qPCR R	TCCGTAGAGTGTGCCATTCATC
gapA qPCR F	CTGCTGAAGGCGAAATGAAAGG
gapA qPCR R	GTAACCAGACTTCGGCACGAATA
	Primers used for Lambda Red Recombinase
sinH λ F	CTGAATAATCAGGGATACAATATTAAAGCTTATTTGTGTAGGCTGGAGCTGCTTC
sinH λ R	CCTTCAGCGACTGGCCGACTGGCCTTACGCCAATCATGGGAATTAGCCATGGTCC
sinI λ F	GGCCAGTCGCTGAAGGTTCATTAAGGAGAGCATCAGTGTAGGCTGGAGCTGCTTC
sinI λ R	CAGCATTCCCTTGCGGGAATGCTGCTGAGTACGCGATGGGAATTAGCCATGGTCC
sinH-I λ F	ACTCTTTATGACATGCCTTTCTGAATAATCAGGGAGTGTAGGCTGGAGCTGCTTC
sinH-I λ R	TTAAGCAAAATATGCGGAAACAGCATTCCCTTGCGATGGGAATTAGCCATGGTCC
ratA λ F	CAAGCGTCCGTTTAACAACCGGTGGAGTTTCTGTCGTGTAGGCTGGAGCTGCTTC
ratA λ R	ATAACTCCCAGGCTACGCCAGATCCGCCATGCTGGATGGGAATTAGCCATGGTCC
	Primers for confirming Lambda Red mutants
sinH gDNA F	TCCGATCAAGCCGAACAAGAA
sinH gDNA R	CCGAGGAAAGTGACATCCACATAG
sinI gDNA F	CCAGTCGCTGAAGGTTCATTAAG
sinI gDNA R	ATGCGGAAACAGCATTCCCTT
sinH-I gDNA F	GACATGCCTTTCTGAATAATCAGGG
sinH-I gDNA R	GACGCTTGTCGCATCAACATAT
ratA gDNA F	ATGTTGATGCGACAAGCGTCC
ratA gDNA R	GCATCGCATCCGGCAATAACT
	Primers used for IE Assay
IE PCR F	AGTAATGCTGCTCGTTTTGC

IE PCR R	GACAGAGCCGACAGAACAAC
	Primers used for Complementation Cloning
pGEN_MCS F	ACACAATCTGCCCTTTCGAA
pGEN_MCS R	CCAGCATGAAGAGTTTCAGA
pGEN_*sinH* F	TCTGAAACTCTTCATGCTGGGCTGTGGATTATTTCTGCCAACATG
pGEN_*sinH* R	TTCGAAAGGGCAGATTGTGTCGACTGGCCGACTGGCCTTACGCCA
pBAD F	GGTTAATTCCTCCTGTTAGCC
pBAD R	GGGCCCGAACAAAAACTCATC
pBAD_*sinH* F	GGTTAATTCCTCCTGTTAGCCTTGACTGTATCATTGTCACAGGGAA
pBAD_*sinH* R	GGGCCCGAACAAAAACTCATCTTTTTTGGTATACAGCACGCGT

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4. Guzman LM, Belin D, Carson MJ, Beckwith J. 1995. Tight regulation, modulation, and high-level expression by vectors containing the arabinose PBAD promoter. J Bacteriol 177:4121-30.