

***Supporting Information***

**CRISPRi System as an Efficient, Simple Platform for Rapid Identification of  
Genes Involved in Pollutant Transformation by *Aeromonas hydrophila***

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This supplementary material contains 9-page document, including 2 tables, 3 figures,  
and this cover page.

**Table S1 Strains and Plasmids Used in This Work**

strain/plasmid /primers	relevant genotype or phenotype	source or reference
<b><i>A. hydrophila</i></b>		
ATCC7966	wild-type	lab stock
~pHQ	wild-type with plasmid pHQ	this study
~pHQGFP	wild-type with plasmid pHQGFP	this study
~T1	strain expressing sgRNA targeting T1 region of <i>gfp</i>	this study
~T2	strain expressing sgRNA targeting T2 region of <i>gfp</i>	this study
~T3	strain expressing sgRNA targeting T3 region of <i>gfp</i>	this study
~NT1	strain expressing sgRNA targeting NT1 region of <i>gfp</i>	this study
~NT2	strain expressing sgRNA targeting NT2 region of <i>gfp</i>	this study
~NT3	strain expressing sgRNA targeting NT3 region of <i>gfp</i>	this study
~ftsZ	strain expressing sgRNA targeting <i>ftsZ</i>	this study
~arsC-1	strain expressing sgRNA targeting <i>arsC-1</i>	this study
~arsC-2	strain expressing sgRNA targeting <i>arsC-2</i>	this study
~arsR2	strain expressing sgRNA targeting <i>arsR2</i>	this study
~arsB	strain expressing sgRNA targeting <i>arsB</i>	this study
~mtrA	strain expressing sgRNA targeting <i>mtrA</i>	this study
~mtrB	strain expressing sgRNA targeting <i>mtrB</i>	this study
~mtrC	strain expressing sgRNA targeting <i>mtrC</i>	this study
~mtrCAB	strain expressing sgRNA targeting <i>mtrCAB</i>	this study
<b><i>E. coli</i></b>		
WM3064	<i>thrB1004 pro thi rpsL hsdS lacZΔM15</i> RP4-1360Δ( <i>araBAD</i> )567Δ <i>dapA1341</i> ::[erm pir(wt)]	lab stock
Turbo	<i>F' proA<sup>+</sup>B<sup>+</sup> lacIq Δ lacZ M15/fhuA2</i> <i>Δ(lac-proAB) glnV gal R(zgb-210::Tn10)Ter<sup>R</sup></i> <i>endA1 thi<sup>-1</sup> Δ(hsdS-mcrB)5</i>	lab stock
<b>Plasmids</b>		
pYYDT	expression vector	lab stock
pHQ	plasmid for CRISPRi system	this study
pHQGFP	constitutive expression of GFP in pHQ	this study
pHQGFP/T1	pHQGFP expressing sgRNA targeting T1 region of <i>gfp</i>	this study
pHQGFP/T2	pHQGFP expressing sgRNA targeting T2 region of <i>gfp</i>	this study
pHQGFP/T3	pHQGFP expressing sgRNA targeting T3 region	this study

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	of <i>gfp</i>	
pHQGFP/NT1	pHQGFP expressing sgRNA targeting NT1 region of <i>gfp</i>	this study
pHQGFP/NT2	pHQGFP expressing sgRNA targeting NT2 region of <i>gfp</i>	this study
pHQGFP/NT3	pHQGFP expressing sgRNA targeting NT3 region of <i>gfp</i>	this study
pHQ/ftsZ	pHQ expressing sgRNA targeting <i>ftsZ</i>	this study
pHQ/arsC-1	pHQ expressing sgRNA targeting <i>arsC-1</i>	this study
pHQ/arsC-2	pHQ expressing sgRNA targeting <i>arsC-2</i>	this study
pHQ/arsR2	pHQ expressing sgRNA targeting <i>arsR2</i>	this study
pHQ/arsB	pHQ expressing sgRNA targeting <i>arsB</i>	this study
pHQ/mtrA	pHQ expressing sgRNA targeting <i>mtrA</i>	this study
pHQ/mtrB	pHQ expressing sgRNA targeting <i>mtrB</i>	this study
pHQ/mtrC	pHQ expressing sgRNA targeting <i>mtrC</i>	this study
pHQ/mtrCAB	pHQ expressing sgRNA targeting <i>mtrCAB</i>	this study

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**Table S2 Primers Used in This Work**

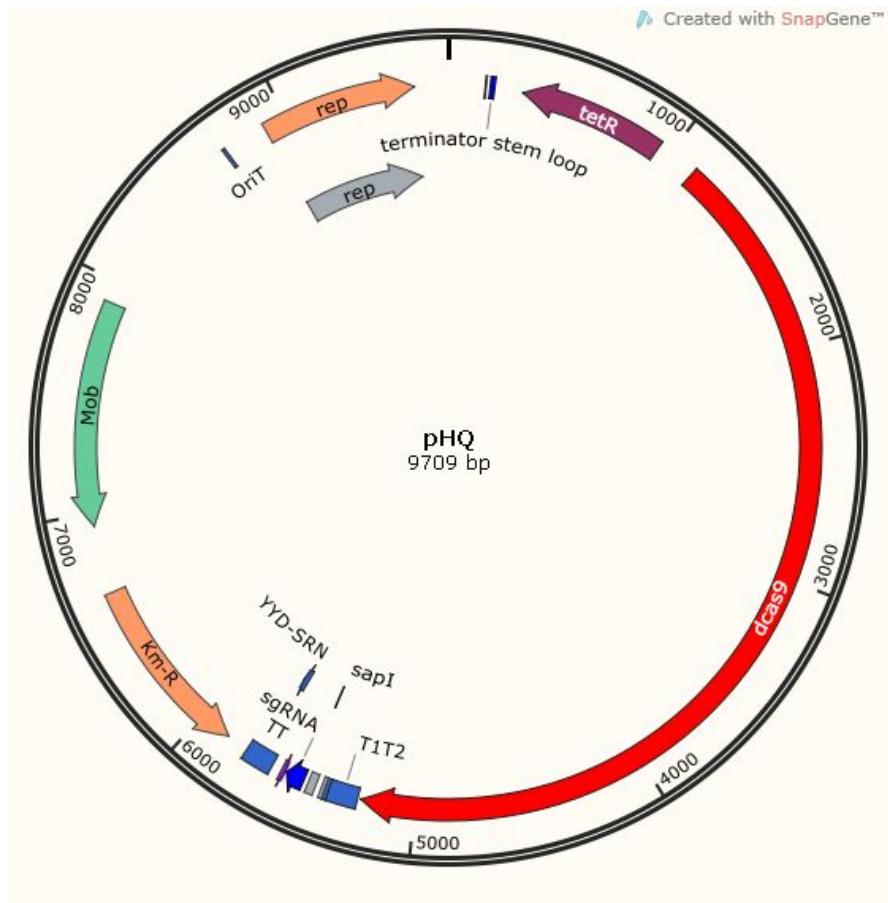
primers	DNA sequences	
<b>plasmid construction</b>		
HQ-1F	ggatgatttcgttgttacctcgga	cloning
HQ-1R	tatataaacgcagaaaggcccacc	TetR-PtetO-dCas9-Tran scription terminator fragment
HQ-2F	ggtgtggcccttctgcgtttatata	cloning the sgRNA
HQ-2R	agggtcgtaaatagccgcattatg	expression cassette
HQ-3F	cataaggcgctatttaacgaccct	cloning pYYDT
HQ-3R	tccgaggttaccacagaaaatcatcc	fragment
HQ-4F	taaaccagcaatagacataagcgcc	cloning
HQ-4R	ttcgaagcaaattcgaccggc	Plac-GFP-Transcription terminator fragment
HQ-5F	gaccgggtcgaatttgcttcgaa	cloning pHQ fragment
HQ-5R	gccgcttatgtctattgctggttt	
T1-1F	Tttccacacaacatacgagcgtttagagctagaaatagcaagtaaaata aggctag	integration of T1 into pHQGFP
T1-1R	gctcgtagttgtgtgaaAgctagcattatacctaggactgagctagc	
T2-1F	catctaattcaacaagaatttttagagctagaaatagcaagtaaaataa ggctag	integration of T2 into pHQGFP
T2-1R	aattcttggtaatttagatggctagcattatacctaggactgagctagc	
T3-1F	tcggagttttgtgataagtttagagctagaaatagcaagtaaaataaag gctag	integration of T3 into pHQGFP
T3-1R	ttatcaacaaaatactccgagctagcattatacctaggactgagctagc	
NT1-1F	cttccggctcgatgtgtgttttagagctagaaatagcaagtaaaataaag gctag	integration of NT1 into pHQGFP
NT1-1R	cacaacatacgagccggaaggctagcattatacctaggactgagctagc	
NT2-1F	aaaggagaagaactttcacgttttagagctagaaatagcaagtaaaataa aggctag	integration of NT2 into pHQGFP
NT2-1R	gtgaaaagttcttccttgcattatacctaggactgagctagc	
NT3-1F	tatcaacaaaatactccgatgttttagagctagaaatagcaagtaaaataa ggctag	integration of NT3 into pHQGFP
NT3-1R	atcggagtatttgttgcattatacctaggactgagctagc	
ftsZ-1F	GAAGCCGTCATTAAAGTGATgttttagagctagaaatag caagtaaaataaggctag	integration of N20 for ftsZ into pHQ
ftsZ-1R	ATCACTTAATGACGGCTTCgtgcattatacctagg ctgagctagc	
arsC-1-1F	CTCCAAGAGCCGTGAAACCCgttttagagctagaaata gcaagtaaaataaggctag	integration of N20 for arsC-1 into pHQ
arsC-1-1R	GGGTTTCACGGCTTGGAGgtgcattatacctagg ctgagctagc	

arsC-2-1F	CATCACCATCTACCACAAACGttttagagctagaaatag caagttaaaaataaggctag	integration of N20 for arsC-2 into pHQ
arsC-2-1R	GGTTGTGGTAGATGGTGATGgctagcattatacctagg actgagctagc	
arsR2-1F	GTCTTCGAGTCTCTGTCGTCgttttagagctagaaatagc aagttaaaaataaggctag	integration of N20 for arsR2 into pHQ
arsR2-1R	GACGACAGAGACTCGAAGACGctagcattatacctagg actgagctagc	
arsB-1F	TAGACCGCAATCTGACCCTCgttttagagctagaaatag caagttaaaaataaggctag	integration of N20 for arsB into pHQ
arsB-1R	GAGGGTCAGATTGCGGTCTAgctagcattatacctagg actgagctagc	
mtrA-1F	TGAAAAAGAACATCCCCGCAGgttttagagctagaaata gcaagttaaaaataaggctag	integration of N20 for mtrA into pHQ
mtrA-1R	CTGCGGGGTGATTCTTTCAgtagcattatacctagg ctgagctagc	
mtrB-1F	AACACTTGGCTCTCCCTGCgttttagagctagaaatag caagttaaaaataaggctag	integration of N20 for mtrB into pHQ
mtrB-1R	GCAGGGAGAGCAAAGTGTGctagcattatacctagg actgagctagc	
mtrC-1F	GTCCTCTTGTGACCCTTAAGgttttagagctagaaatagc aagttaaaaataaggctag	integration of N20 for mtrC into pHQ
mtrC-1R	CTTAAGGGTCAAAAGAGGACgctagcattatacctagg actgagctagc	
HQ-6F	cgcttatgtctattgctggttaccgg	cloning pHQ fragment
HQ-6R	tatttaacgcacctgccctgaaccga	
mtrCAB-1F	ggtaaaccagcaatagacataagcgctagattcacttatctggccctg AC	integration of N20 for mtrCAB into pHQ
mtrCAB-1R	cggttcaggcaggcgttaatagagcgctttaagcttaattcgatcat ta	
<b>RT-PCR</b>		
RT-recA	CAAAC TGACCGCCAACAT GAACGGAGGC GTAGAACTT	this study
RT-gfp	TGCTTGCGAGATACCCA CGTGTCTGTAGTTCCCGTC	this study
RT-arsC-1	GGATGTGGTGCTCTATCTGG CCTTGTAGAGTTCTCCTTGGT	this study
RT-arsC-2	GTTGAACCTACCGTTATCCACT AGTCCCAGCGCCTCATAG	this study
RT-arsR2	CGACATTGCTCTAACACCG CAGCACTCTCGGTGAGATAA	this study

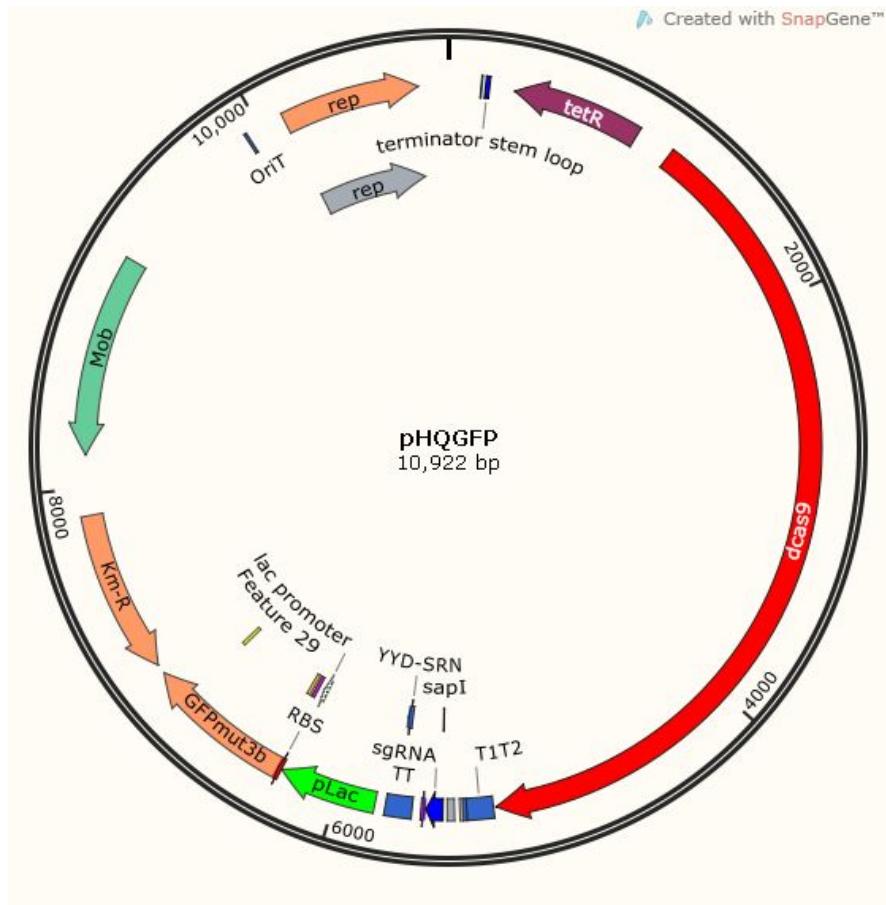
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RT-arsB	CGTTTATTCCGGCAATT ACCAAGGGTACAGCGACC	this study
RT-arsA	TGTGACAGTGGCAAACGG AATCTCCAGTGCTGATAACCC	this study
RT-arsD	AGAAGGGCTGCCTGTGAT GCTGCTGGCTGTTCAACTT	this study
RT-mtr A	AGCAGAACAGCGTCTGTCTC CTGCAGCTTGCTGTCCAT	this study
RT-mtr B	CTATGACTACCCGGATCTAACCC GGAAGTATTCTGTAACGCACAT	this study
RT-mtr C	CCCTGTCATCTGGAAGAGC TTGAAGGAGAAGGTGTAGCG	this study

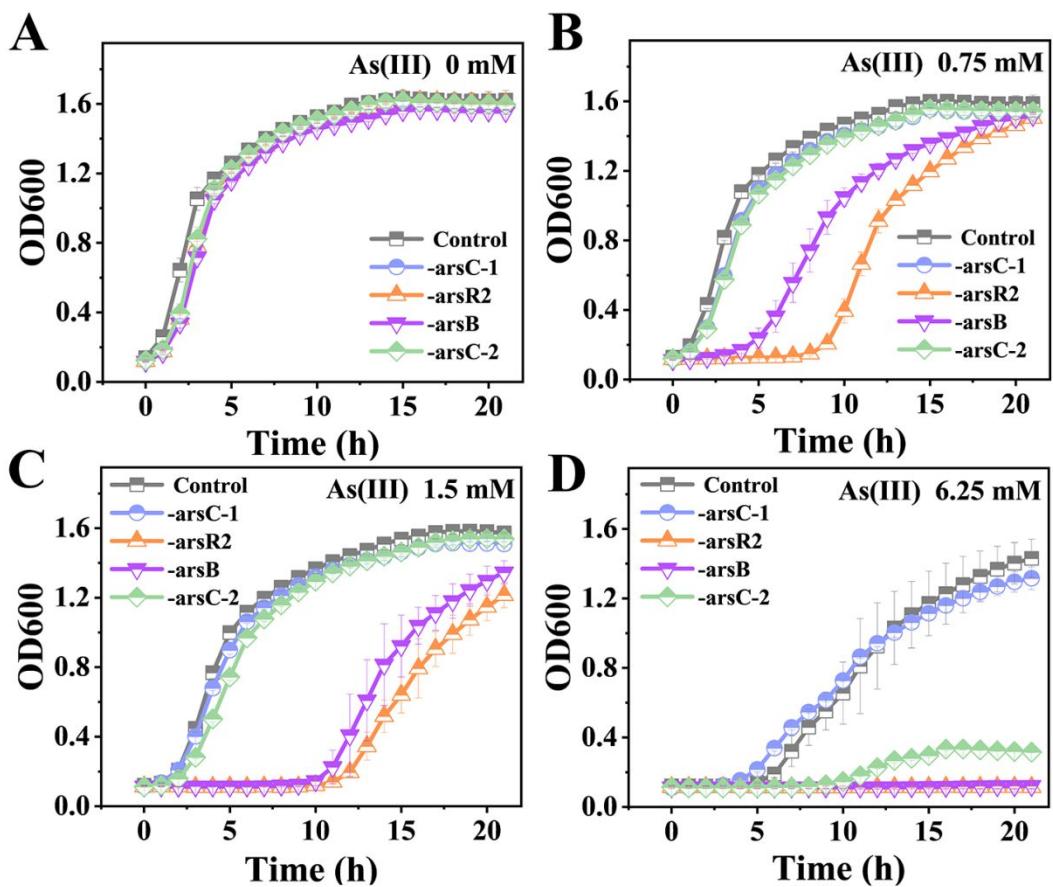
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**Figure S1** Plasmid map of pHQ.



**Figure S2** Plasmid map of pHQGFP.



**Figure S3** Growth of the control and four engineered strains in the presence of 100 ng/ml ATc and 0-6.25 mM As(III).