Figure S1

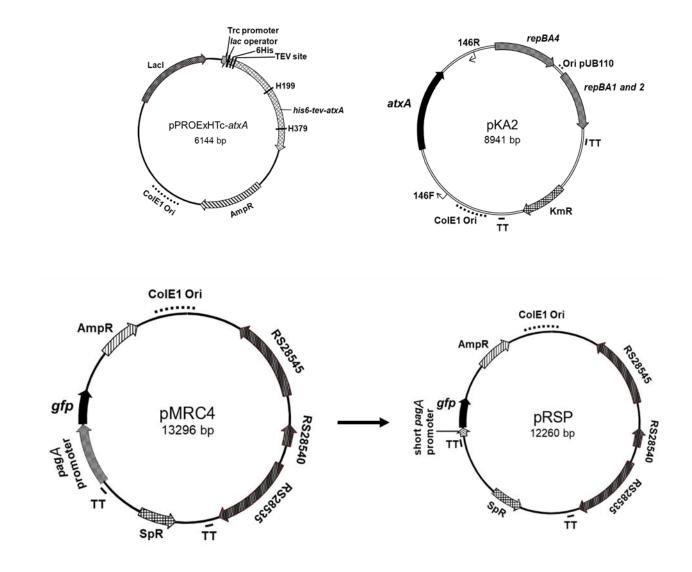
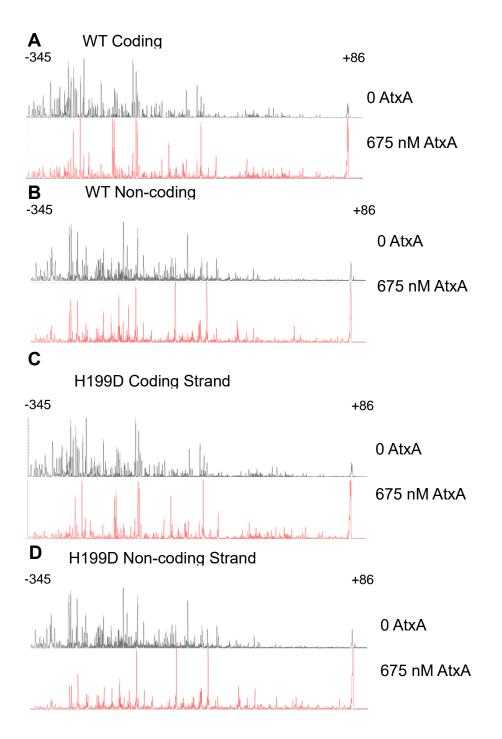
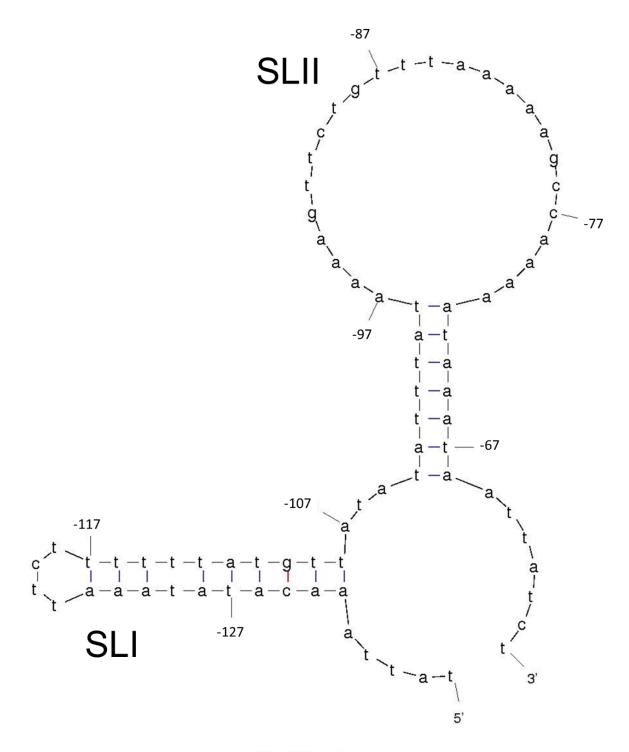


Figure S1. Plasmid pPROExHTc-*atxA*, pKA2, pMRC4 and pRSP maps. Plasmid pPROExHTc-*atxA* was used as a source for purification of native AtxA and its H199A, H199D, H379A,
H379D mutants. Plasmids pKA2 encoding either the native *atxA* gene or its mutant forms
(H199A, H199D, H379A, H379D) were used for complementation of *atxA* in Ames 35ΔAtxA
strain and for activation of *gfp* expression controlled by full length *pagA* promoter in pMRC4 or
short *pagA* promoter in pRSP plasmid. TT – transcription terminators, RS28535-28545 represent
ORFs of pXO1 minireplicon.

8 Figure S2



9	Figure S2. DNase I Footprinting analysis of the entire PAG4 fragment. Footprinting was done
10	with FAM/VIC end-labeled fragments generated using labeled primers in PCR amplification.
11	Mixtures of protein and DNA were incubated with 0.07 U of DNase I for 10 min at room
12	temperature. Reactions were heat inactivated, purified, and sent to OSU-PMGF for analysis on
13	the 3730 DNA Analyzer. Figure shows resulting peaks generated by incubation of the PAG4
14	fragment both with and without protein. (A) Coding strand of PAG4 incubated with AtxA WT.
15	(B) Non-coding strand of PAG4 incubated with AtxA WT. (C) Coding strand of PAG4
16	incubated with AtxA H199D. (D) Non-coding strand of PAG4 incubated with AtxA H199D.



dG = -4.21 pagA prom

- 17 Figure S3. Predicted secondary structure of part of *pagA* promoter containing stem loops. Figure
- 18 displays basepairs -137 to -59 from transcriptional start site and each of the hypothesized stem
- 19 loops are labeled. This structure contains the lowest Gibbs Free Energy of all possible structures
- 20 for this region and was created using the mfold web server
- 21 (http://unafold.rna.albany.edu/?q=mfold/DNA-Folding-Form).

Figure S4

Bacillus anthracis str. 'Ames Ancestor' plasmid pXO1, complete sequence Sequence ID: <u>AE017336.2</u>

Range 1: 143622 to 143662 GenBank Graphics Vext Match 🔺 Previous Match					
Score 76.8 bits(41)	Expect 4e-14	Identities 41/41(100%)	Gaps 0/41(0%)	Strand Plus/Plus	
Query 1	ΑΑCΑΤΑΤΑΑΑΤΤCTTT	TTTATGTTATATATTTATA	AAAGTT 41		
Sbjct 143622		TTTATGTTATATATTTATA	 AAAGTT 143662		

Bacillus anthracis str. Sterne plasmid pXO1, complete sequence Sequence ID: <u>CP009540.1</u> Length: 181624 Number of Matches: 1

Range 1: 31793 to 31833 GenBank Graphics Vext Match 🔺 Previous Mat						
Score	Expect	Identities	Gaps	Strand		
75.2 bits(82)	4e-11	41/41(100%)	0/41(0%)	Plus/Minus		
Query 1 AACATATAAATTCTTTTTTATGTTATATATTTATAAAAGTT 41						
Sbjct 31833	AACATATAAATTCTT	ITTTATGTTATATATTTATA	AAAGTT 31793			

Bacillus cereus G9241 plasmid pBCX01, complete sequence Sequence ID: <u>CP009592.1</u> Length: 190860 Number of Matches: 1

Range 1: 83110 to 83151 GenBank Graphics Vext Match 🔺 Previous Match					
Score 68.9 bits(75)	Expect 6e-09	Identities 41/42(98%)	Gaps 1/42(2%)	Strand Plus/Plus	
Query 1	ΑΑCΑΤΑΤΑΑΑΤΤCTTT		AAAGTT 41	1100/1100	
Sbjct 83110		TTATGTTATATATTTATA			

Bacillus cereus biovar anthracis str. CI plasmid pCI-XO1, complete sequence Sequence ID: <u>CP001747.1</u> Length: 181907 Number of Matches: 1

Range 1: 143876 to 143917 GenBank Graphics Tevrious Ma						ous Match
Score		Expect	Identities	Gaps	Strand	
68.9 b	oits(75)	6e-09	41/42(98%)	1/42(2%)	Plus/Plus	
Query	1	AACATATAAATTCTTT	T-TTATGTTATATATTTAT	AAAAGTT 41		
Sbjct	143876	AACATATAAATTCTTT	TTTTATGTTATATATTTAT	AAAAGTT 143917		

Figure S4. BLAST analysis of DNA region responsible for *pagA* promoter activation.

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