Rational design of an artificial genetic switch: co-option of the H-NS-repressed *proU* operon by the VirB virulence master regulator.

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SUPPLEMENTARY DATA FILE

Table S1: Primers

Oligonucleotides	Sequence (5' to 3') [a] [b]
icsB fw	tgtacctcgtgagcatatgtagt
icsB rev	ggggcattgattgcagtttt
*pro*U rev +230.XbaI	ttttctagatatctcgccttcttcaatgg
*pro*U fw -270.NotI	tttgcggccgcctacccgcagcagggaaata
*pro*U fw -150.NotI	tttgcggccgctcgctatctttgacaaaaatatc
proU fw -60.NotI	tttgcggccgcgccacatttgccatcaggg
proU SDM FseI fw	tttgctcgcatcaatattcaGGCCGGCCttgccatcaggggttgcctc
proU SDM FseI rev	gaggcaacccctgatggcaaGGCCGGCCtgaatattgatgcgcaaa
yaeT fw.SmaI	tttcccgggaagaacgtaacactggtagct
yaeT rev. NotI	tttgcggccgcgacaggtcggcgtcatctg
tet.40 bp *proU* fw	aagactggaatttctgagggtgttattttcaaaatatctcattaagacccactttcacatt
tet .40 bp *proU* rev	cgcggcactgtacgccgctggcgtggtatcccacggattactaagcagttgtctcctg
icsB.fw.40 bp *proU*	aagactggaatttctgagggtgttattttcaaaatatctgtacctcgtgagcatatgt
icsB.rev.40 bp *proU*	aagactggaatttctgagggtgttattttcaaaatatcgggggattgattgcagtttt
cat.rev.40 bp *proU*	cgcggcactgtacgccgctggcgtggtatcccacggattacacttattcaggcgtagcag
rho.RT.fw	gttgtagtcgatagccgcga
rho.RT.rev	tacaggcaacatggaactgc
P1.RT.fw	cttatatcacgggaaattcc
P2.RT.fw	gggtaatatatcgacatagac
gfp.RT.rev	agtgaaaagttcttctcctt

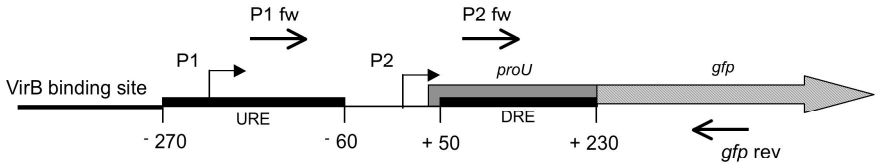
[a] Underlined nucleotides represent restriction enzyme sites used for cloning

[b] Capitol letters indicated base pair substitutions made in site-directed mutagenesis

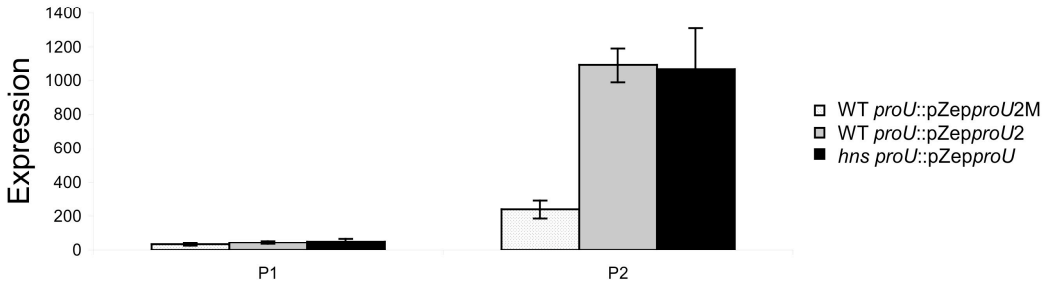
VirB dependent de-repression of *proU* is mediated by promoter P2 and not P1. To determine which promoter was responding to VirB mediated de-repression, qRT-PCR was preformed with the chromosomal integrations of pZep-*proU*-1, pZep-*proU*-2M and pZep-*proU*-2. These hybrid promoters were inserted into the native *proU* locus in both wild type and *hns* strains of *S. flexneri*. To differentiate between transcripts originating at the *proU* P1 and P2 promoters, forward primers specific for detection of transcripts from either P1 (P1 fw) or P1 and P2 combined (P2 fw) were used (Fig. S1A). The results from both P1 fw and P2 fw primers confirmed that expression from the VirB de-repressed *proU* was mediated by the P2 promoter and not the adjacent P1 promoter. Just as had been seen in the plasmid-based experiments, constructs with the VirB binding site at position -270 (pZep-*proU*-2) of the *proU* P2 promoter was derepressed in the *S. flexneri* wild type background to levels similar to those seen in the *hns* mutant. With the mutated, non-functional VirB binding site inserted at the same position (pZep-*proU*-2M), VirB dependent derepression was not observed (Fig. S1B).

FIG S1. Transcription from derepressed *proU* promoters is mediated by promoter P2, not P1. A. Schematic of pZep-*proU*-2, the modified *proU* promoter that was integrated into the *proU* locus on the chromosome. Primers P1 fw, P2 fw and *gfp* rev are indicated with arrows. B. Quantitative PCR measurements of gene transcript levels (arbitrary values) from promoter P1 (primer sets P1 fw and *gfp* rev), and promoter P2 (P2 fw and *gfp* rev). Measurements were taken from wild type cells with pZep-*proU*-2M (mutated binding site at position -270) inserted, wild type cells with the pZep-proU-2 (functional VirB binding site at position -270) inserted, and *hns* cells also haboring pZep-proU-2 in place of native *proU*. All experiments were conducted with bacteria grown under repressive conditions in 0 mM NaCl growth medium.

A.



B.



Supplementary Data: Figure S1