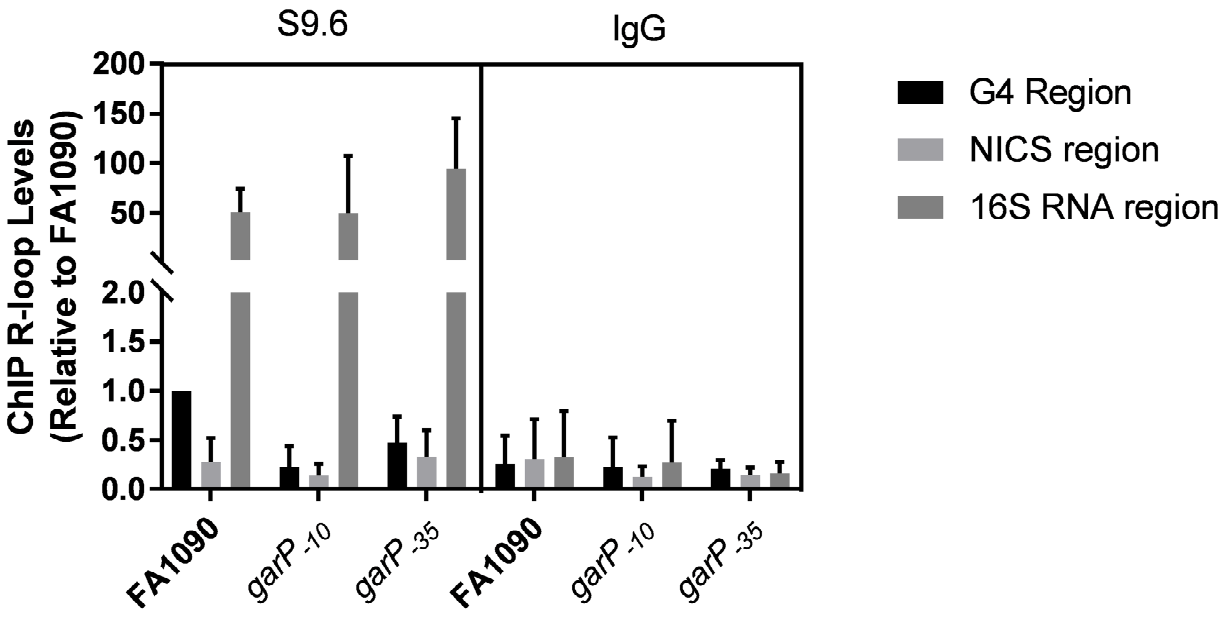
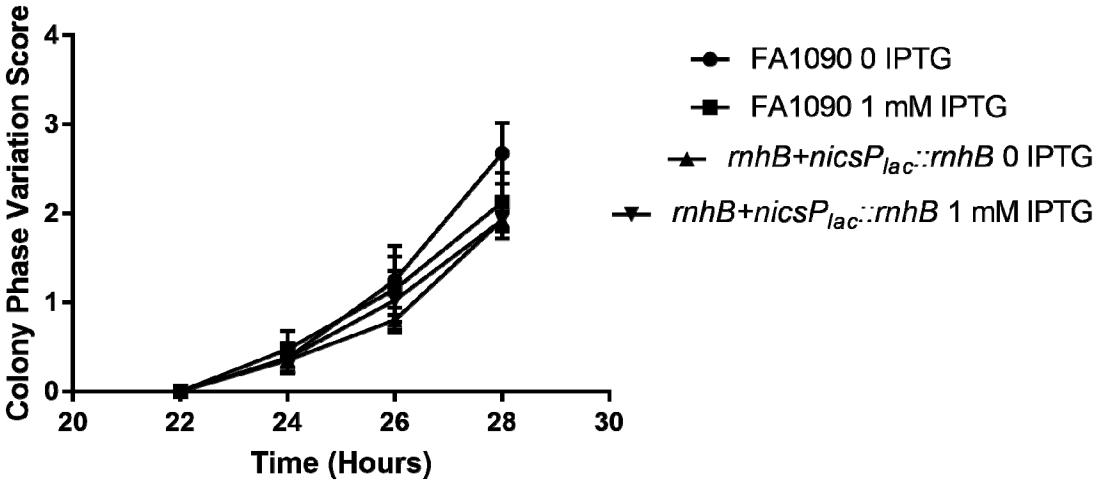
Supplemental Information

Contents



Supplemental Figure 1. R-loop ChIP levels with *garP* mutations

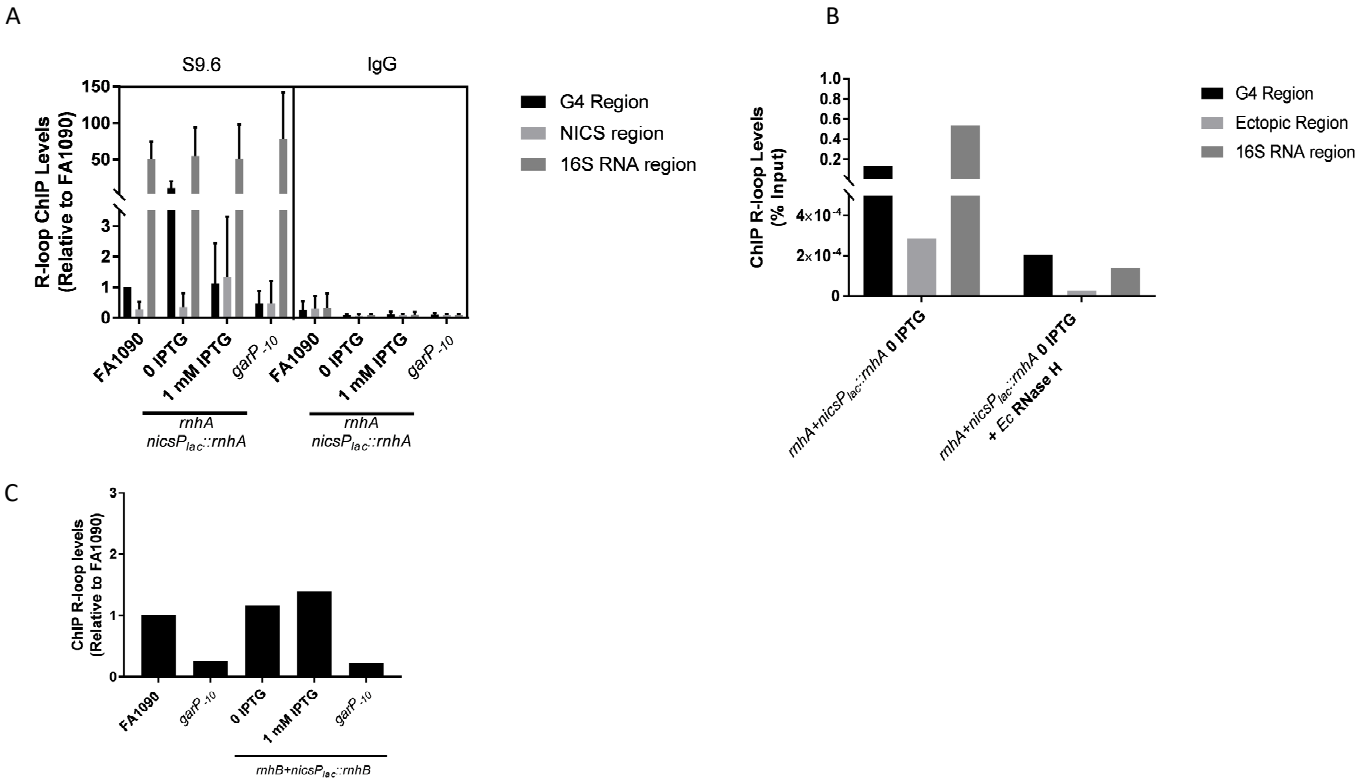
Graphs represents the same data as Figure 2A, but the negative and positive controls are also included. The ectopic region is a low transcription region of the genome and ChIP signal detects general genomic DNA pulled down by the S9.6 antibody and 16S RNA region is a region of high transcription so this is a positive control for R-loop pull down for each experiment. A nonspecific IgG Ab indicates the general DNA pulled down by a nonspecific antibody.



Supplemental Figure 2. *rnhB* does not affect pilus dependent colony morphology changes

Phase variation of FA1090 and *rnhB+nicsP*$_{lac}$*::rnhB* was determined using the PDCMC assay with and without 1 mM IPTG. The average of 4 biological replicates with standard deviation is graphed.

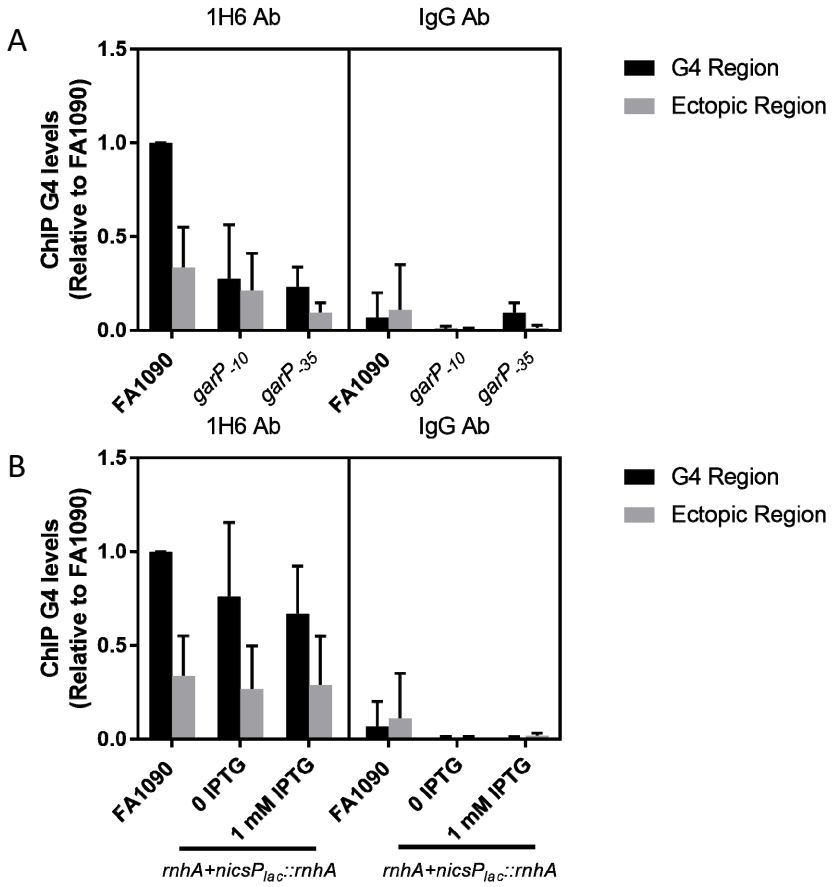
Supplemental Figure 3.



Supplemental Figure 3. R-loop ChIP controls

A. Graphs represents the same data as 2C but negative and positive controls are also included. The ectopic region is a low transcription region of the genome and ChIP signal detects general genomic DNA pulled down by the S9.6 antibody and 16S RNA region is a region of high transcription so this is a positive control for R-loop pull down for each experiment. A nonspecific IgG Ab indicates the general DNA pulled down by a nonspecific antibody.
B. R-loop ChIP was performed on *rnhA*+*nicsP*$_{lac}$*::rnhA* 0 IPTG. The DNA bound to S9.6 was treated with *E. coli* RNase H before washing (50). The resulting DNA was purified and % Input calculated. Average of two experiments is graphed. The on bead treatment with purified RNase H should remove the R-loop signal indicating the S9.6 antibody is detecting RNA:DNA hybrids.
C. R-loop ChIP was used to determine the level of R-loops in *rnhB*+*nicsP*$_{lac}$*::rnhB* with and without the addition of IPTG. The average of two experiments is graphed. The range for *rnhB*+*nicsP*$_{lac}$*::rnhB* 0 IPTG was 0.2-2, 1 mM IPTG 0.6-2.2 and the *rnhB*+*nicsP*$_{lac}$*::rnhB garP*$_{-10}$ 0.04-0.4 fold.

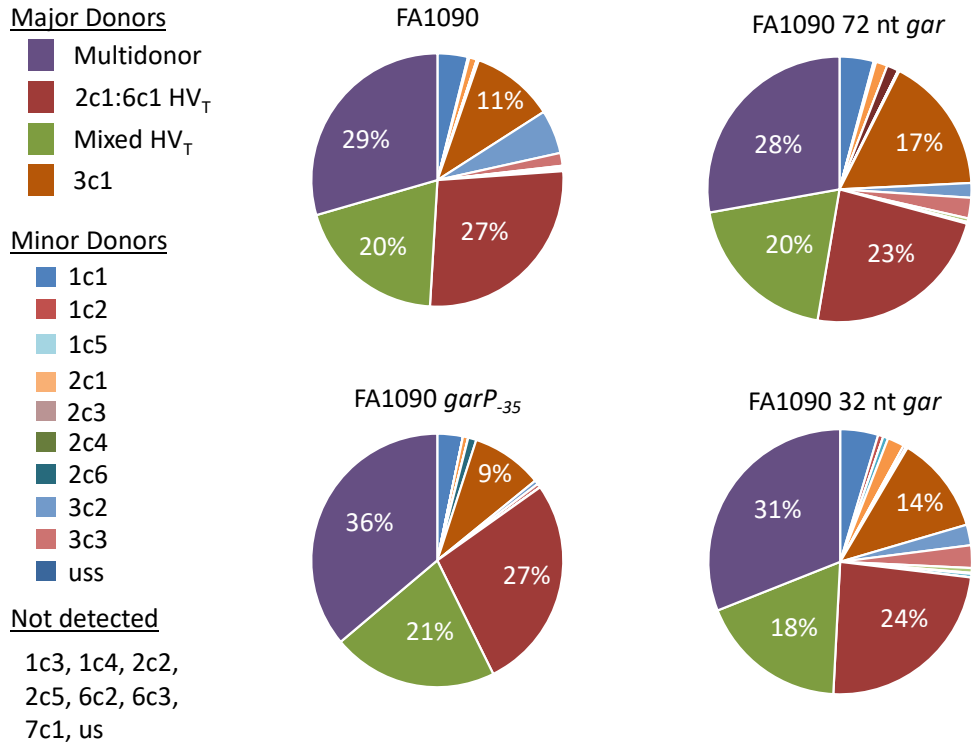
Supplemental Figure 4.



Supplemental Figure 4. G4 ChIP Controls

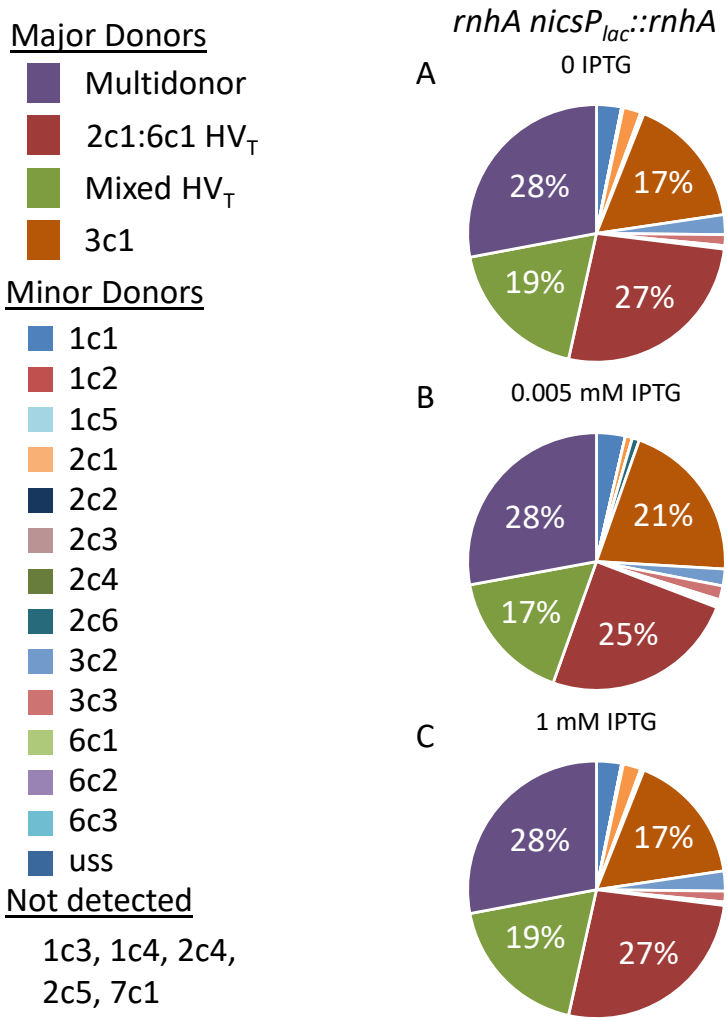
A. G4 levels were determined using G4 ChIP on *garP*$_{-10}$ and *garP*$_{-35}$ with the G4 specific Ab, 1H6. Relative G4 levels are calculated by determining the % input after ChIP pulldown for each sample and the dividing by the % input of FA1090. Graph represents the same data as Figure 4A. The ectopic region is a low transcription region of the genome and ChIP signal detects general genomic DNA pulled down by the antibody. A nonspecific IgG Ab indicates the general DNA pulled down by a nonspecific antibody.
B. G4 ChIP was used to determine G4 levels under low *rnhA* and high *rnhA* expression (*rnhA*+*nicsP*$_{lac}$*::rnhA* 0 IPTG, and *rnhA*+*nicsP*$_{lac}$*::rnhA* 1 mM IPTG). The data is the same as Figure 4B. The controls are the same as described in part A.

Supplemental Figure 5.



Supplemental Figure 5. Silent copy choice for pilin Av events detected using PacBio and SwitchAmp.

The two biological replicates were pooled and all the silent copies chosen for each pilin Av counted. There are four common silent copy choices seen in the samples. The tail sequence of silent copies 2c1 and 6c1 (2c1:6c1 HV$_T$) replaced the *pilE* sequence in many samples, similar to previously published results (Criss *et al.*, 2005, Rotman *et al.*, 2016). We also observed a mosaic sequence containing both 1-81-S2 and the 2c1:6c1 tail sequence that was not observed in the negative control strains. Additionally, the use of silent copy 3c1 was common among all the strains. Finally, the fourth choice was multivariant, where the change way common to multiple silent copies so a single donor cannot be identified (Ozer, *et al.* 2019).



Supplemental Figure 6. Silent copy choice during different levels of *rnhA* expression

The silent copy choice was examined for *rnhA+nicsP$_{lac}$::rnhA* with 0, 0.005 and 1 mM IPTG from the two biological replicates. The four common silent copy choices were similar to Supplemental Figure 5.

	Pool A		Pool B	
Strain	**% Av**	**Reads**	**%Av**	**Reads**
FA1090	17.61	7296	16.9	5396
FA1090 G4 Mutant	0.21	7252	ND	ND
FA1090 *garP*$_{-10}$	0.1	4707	ND	ND
FA1090 *garP*$_{-35}$	6.13	4522	5.73	4434
FA1090 72 nucleotide *gar*	17.60	6018	13.7	6312
FA1090 32 nucleotide *gar*	16.35	7164	16.75	6873
rnhA+*nicsP*$_{LAC}$*::rnhA* 0 IPTG	11.9	7667	7.38	5327
rnhA+*nicsP*$_{LAC}$*::rnhA* 0.005 mM IPTG	12.6	6098	13.9	7290
rnhA+*nicsP*$_{LAC}$*::rnhA* 1 mM IPTG	12.8	6297	12.3	5039
rnhA+*nicsP*$_{LAC}$*::rnhA garP*$_{-10}$ 0 IPTG	0.19	8965	ND	ND

Supplemental Table 1. Pilin Av frequencies by the PacBio Assay

Each strain that can undergo pilin Av was tested with biological replicates. The pool of both replicates for each sample are shown in Table 1. This table contains the individual values of each pool separately, and includes the number of reads for each pool. The Av frequency was determined by dividing the number of variant reads by the total number of reads per barcoded sample. ND-not determined

Supplemental Table 2.

Sample name	Condition	Rep #	Library ID	SRA accession	BioSample accession	# Reads	# Bases	Mean read length
35mutFA_22h_a	FA1090 garP-35	1	33a	SRR9670522	SAMN12255025	5972	4717392	789.92
35mutFA_22h_b	FA1090 garP-35	2	33b	SRR9670511	SAMN12255041	6011	4577275	761.48
FA-10mut_22h	FA1090 garP-10 22 hrs		31b	SRR9670517	SAMN12255039	6015	4759857	791.33
FA1090_22h_a	FA1090 grown for 22 hrs	1	1a	SRR9670503	SAMN12255012	10842	8628492	795.84
FA1090_22h_b	FA1090 grown for 22 hrs	2	1b	SRR9670527	SAMN12255026	8690	6941742	798.82
G4mutFA1090_22h	FA1090 G4 mutant 22 hrs		23b	SRR9670514	SAMN12255034	8757	6925157	790.81
endterm_22h_a	FA1090 32 nt gar 22 hrs	1	18a	SRR9670505	SAMN12255018	10625	8392303	789.86
endterm_22h_b	FA1090 32 nt gar 22 hrs	2	18b	SRR9670516	SAMN12255032	10449	8284712	792.87
116term_22h_a	FA1090 72 nt gar 22 hrs	1	17a	SRR9670508	SAMN12255017	10597	8436107	796.08
116term_22h_b	FA1090 72 nt gar 22 hrs	2	17b	SRR9670530	SAMN12255031	10387	8307828	799.83
delA+A_32h_a	FA1090 rnhA+nicsPlac::rnhA 0 mM IPTG 32 hrs	1	8a	SRR9670507	SAMN12255016	10576	8403174	794.55
delA+A_32h_b	FA1090 rnhA+nicsPlac::rnhA 0 mM IPTG 32 hrs	2	7b	SRR9670526	SAMN12255029	7387	5698879	771.47
delA+ALo_22h_a	FA1090 rnhA+nicsPlac::rnhA 0.005 mM IPTG 22 hrs	1	6a	SRR9670501	SAMN12255014	9895	7853573	793.69
delA+ALo_22h_b	FA1090 rnhA+nicsPlac::rnhA 0.005 mM IPTG 22 hrs	2	5b	SRR9670528	SAMN12255027	11280	8595019	761.97
delA+AHi_22h_a	FA1090 rnhA+nicsPlac::rnhA 1 mM IPTG 22 hrs	1	7a	SRR9670502	SAMN12255015	9574	7586886	792.45
delA+AHi_22h_b	FA1090 rnhA+nicsPlac::rnhA 1 mM IPTG 22 hrs	2	6b	SRR9670525	SAMN12255028	8330	6565278	788.15
delA+A-10mut_32h	FA1090 rnhA+nicsPlac::rnhA garP-10 0 mM IPTG 32 hrs		8b	SRR9670529	SAMN12255030	10573	8253947	780.66

Supplemental Table 2. PacBio samples numbers

The strains analyzed in this study along with their read statistics and accession numbers. Reads can be accessed under the SRA project SRP214219 with the Bioproject accession number PRJNA553228.

recA6 FA1090	(Seifert 1997)
FA1090	Lab strain
garP$_{-10}$ recA6 FA1090	(Cahoon and Seifert 2013)
garP$_{-35}$ recA6 FA1090	(Cahoon and Seifert 2013)
82sRNA *recA6* FA1090	This study
72sRNA FA1090	This study
32sRNA FA1090	This study
recQ/rep recA6 FA1090	(Cahoon and Seifert, 2009)
G4mutant *recA6* FA1090	This study
G4mutant FA1090	This study
rnhA+nicsP$_{lac}$::rnhA FA1090	This study
rnhA+nicsP$_{lac}$::rnhA garP$_{-10}$ FA1090	This study
garP$_{-10}$ FA1090	This study
garP$_{-35}$ FA1090	This study

Supplemental Table 3. Strains used in this study
Descriptions of how each strain was constructed can be found in the methods.

Supplemental Table 4. Primers used in this study

rnhA-usF	GTCCGCAGCCCATATCC
rnhA-dsR	TCGCATACCGGATAAGGGC
rnhAusR-NotI	TGTCGGTGCGGCAATCAGCGGCCGCATCGTGCCTTTGTGTGGTG
rnhAdsF-NotI	CACCACACAAAGGCACGATGCGGCCGCTGATTGCCGCACCGACA
rnhAF-PacI	GGCttaattaaATGGACACACCCGTTTACC
rnhAr-PmeI	GGCgtttaaacTCGCATACCGGATAAGGGC
R2usF	GAAGACAACGCCGATATGCG
R2dsR	TGCTGAAGCACCAAGCGAAC
R2usRkpnI	ACGGCATTTTTGTGCCGGTTTAggtaccCGCACCGATGTGTTTATTTCG
R2dsFkpnI	CGAAATAAACACATCGGTGCGgtaccTAAACCGGCACAAAAATGCCGT
R2FPacI	GGTttaattaaATGCCGTCTGAAACCATTTTC
R2dsRFseI	GGTggccggccTGCTGAAGCACCAAGCGAAC
RT-rnhAF2	CGTCATCATCTGCACCGACT
RT-rnhAR2	TCTTGCCACAAGTCGTCGTT
RTG4-3F	AAATCGGCACGAATCTTGCTT
RTG4-3R	TCAGCTCGATAAGGGTAAAGCC
32nt sRNA gBlock	ATACTTAATTAAGCATAGAAACACCACGCGCCGATTTCAAATGCTTTCCAAGA AAACGGAGCGAGTCGAAAAAAAAGCCCGCTCATTAGGCGGGCAACTGTGTGT TTTTTTAAAAAATAAAAAATTCCCCACCCAACCCACCCTATTCTAACGCGTAAA TTCAAAAATCTCAAATTCCGACCCAATCAACACACCCGATACCCCATGCCAATA AAAAAGTAACGAAAATCGGCACTAAAACTGACAATTTTCGACACTGCCGCCCC CTACTTCCGCAAACCACACCCACCTAAAAGAAAATACAAAATAAAAACAATTA TATAGAGATAAACGCATAAAATTTCACCTCAAAACATAAAATCGGCACGAATC TTGCTTTATAATACGCAGTTGTCGCAACAAAAAACCGATGGTTAAATACATTG CATGATGCCGATGGCGTAAGCCTGAGGCATTTCCCCTTTCGCCGTCTGAACC
72nt sRNA gBlock	ATACTTAATTAAGCATAGAAACGAGTCGAAAAAAAAGCCCGCTCATTAGGCG GGCAACTGTGTGTTACCACGCGCCGATTTCAAATGCTTTCCAAGAAAACGGAG CTTTTTAAAAAATAAAAAATTCCCCACCCAACCCACCCTATTCTAACGCGTAAA TTCAAAAATCTCAAATTCCGACCCAATCAACACACCCGATACCCCATGCCAATA AAAAAGTAACGAAAATCGGCACTAAAACTGACAATTTTCGACACTGCCGCCCC CTACTTCCGCAAACCACACCCACCTAAAAGAAAATACAAAATAAAAACAATTA TATAGAGATAAACGCATAAAATTTCACCTCAAAACATAAAATCGGCACGAATC TTGCTTTATAATACGCAGTTGTCGCAACAAAAAACCGATGGTTAAATACATTG CATGATGCCGATGGCGTAAGCCTGAGGCATTTCCCCTTTCGCCGTCTGAACC

84nt sRNA gBlock	ATATTAATTAAGAGTCGAAAAAAAAGCCCGCTCATTAGGCGGGCAACTGTGT GTTGCATAGAAACACCACGCGCCGATTTCAAATGCTTTCCAAGAAAACGGAGC TTTTTAAAAAATAAAAAATTCCCCACCCAACCCACCCTATTCTAACGCGTAAAT TCAAAAATCTCAAATTCCGACCCAATCAACACACCCGATACCCCATGCCAATAA AAAAGTAACGAAAATCGGCACTAAAACTGACAATTTTCGACACTGCCGCCCTT CAGACGGCGGTACCACCCACCTAAAAGAAAATACAAAATAAAAACAATTATA TAGAGATAAACGCATAAAATTTCACCTCAAAACATAAAATCGGCACGAATCTT GCTTTATAATACGCAGTTGTCGCAACAAAAAACCGATGGTTAAATACATTGCA TGATGCCGATGGCGTAAGCCTGAGGCATTTCCCCTTTCAATTAGGAGTAATTT
16sF1	GGAGACGGAGGAGTGCCTTC
16sR1	CGCTCGTTGCGGGACTTAAC
USaspcF1	GTCCGGTCCCGAGCAATACA
USaspcR1	TAGCCTGCCGATGGCGTAAA
RT-G4-start	GGGTTGGGTGGGGAATTTTT
RT-mid-For	GCTTTCCAAGAAAACGGAGC
RT-Term-For	ATCTTGTGCAATGTAACATCAGAG
KanFor	ATGGCTCATAACACCCCTTG
lctpout1F1	catcatcgccgtatgtaccg
LacPFor	gaggcgataacaatttcaca
garP-10	TGAACCAACTGCCACCTAAGGCAAATTAGGCCTTAAATTTCAAATAAATCAAA CGGTAAGTGATTTTCCACGGCCGCCCGGATCAACCCGGGCGGCTTGTCTTTTA AGGGTTTGCAAGGCGGGCGGGGTCGTCCGTTCCGAAGCCATCCTTTTGGCCG AAGGTCAAAAATCAGCCGTTACCGGGTATTGCCCGAATCACGGCATATGGCC GGAAAACTTCGTCATTCCCGCGAAAGCGGGAATCTAGGTCTGTCGGCACGGA AACTTATCGGGTAAAAAGGTTTCTCCGGTCCTGAGTCCTGGATTCCCACTTTCG TGGGAATGACGGGATTTAATGATGCCGCCGGCAACGAAAAAATCGAAACCAA GCACCTGCCGTCAACCTGCCGCGACGCTTCATCTGCCGGTTGCATAGAAACAC CACGCGCCGATTTCAAATGCTTTCCAAGAAAACGGAGCTTTTTAAAAAATAAA AAATTccccacccaacccacccTggacggACGCGTAAATTCAAAAATCTCAAATTCCG ACCCAATCAACACACCCGATACCCCATGCCAATAAAAAAGTAACGAAAATCGG CACTAAAACTGACAATTTTCGACACTGCCGCCCCCTACTTCCGCAAACCACACC CACCTAAAAGAAAATACAAAATAAAAACAATTATATAGAGATAAACGCATAA AATTTCACCTCAAAACATAAAATCGGCACGAATCTTGCTTTATAATACGCAgTT GTCGCAACAAAAAACCGATGGTTAAATACATTGCATGATGCCGATGGCGTAA GCCTGAGGCATTTCCCCTTTCAATTAGGAGTAATTTTATGAATACCCTTCAAAA AGGCTTTACCCTTATCGAGCTGATGATTGTGATCGCTATCGTCGGCATTTTGGC GGCAGTCGCCCTTCCCGCCTACCAAGACT

G4mut gBlock	ATACTTAATTAAGCATAGAAACACCACGCGCCGATTTCAAATGCTTTCCAAGA AAACGGAGCTTTTTAAAAAATAAAAAATTCCCCACCACACCCCCACTATTCTAA CGCGTAAATTCAAAAATCTCAAATTCCGACCCAATCAACACACCCGATACCCC ATGCCAATAAAAAAGTAACGAAAATCGGCACTAAAACTGACAATTTTCGACAC TGCCGCCCCCTACTTCCGCAAACCACACCCACCTAAAAGAAAATACAAAATAA AAACAATTATATAGAGATAAACGCATAAAATTTCACCTCAAAACATAAAATCG GCACGAATCTTGCTTTATAATACGCAGTTGTCGCAACAAAAAACCGATGGTTA AATACATTGCATGATGCCGATGGCGTAAGCCTGAGGCATTTCCCCTTTCGCCG TCTGAACC

Supplemental Table 4. Primers used in this study

Primers and gBlocks were used to construct strains or in quantitative PCR assays to quantify DNA.

Supplemental Table 5. Primers used for PacBio Sequencing

Barcodes for PacBio Sequencing				Strain
bc1002pilRBS	ACACACAGACTGTGAGtttccccctttcaattaggag	bc1002opaeRev	ACACACAGACTGTGAGgggttccgggcggtgtttc	FA1090
bc1010pilRBS	ACGCGCTATCTCAGAGtttccccctttcaattaggag	bc1010opaeRev	ACGCGCTATCTCAGAGgggttccgggcggtgtttc	*rnhA*+*nicsP*$_{lac}$::*rnhA* 0.005 mm IPTG
bc1011pilRBS	CTATACGTATATCTATtttccccctttcaattaggag	bc1011opaeRev	CTATACGTATATCTATgggttccgggcggtgtttc	*rnhA*+*nicsP*$_{lac}$::*rnhA* 1 mm IPTG
bc1012pilRBS	ACACTAGATCGCGTGTtttccccctttcaattaggag	bc1012opaeRev	ACACTAGATCGCGTGTgggttccgggcggtgtttc	*rnhA*+*nicsP*$_{lac}$::*rnhA* 0 mm IPTG
bc1027pilRBS	CTCACACTCTCTCACAtttccccctttcaattaggag	bc1027opaeRev	CTCACACTCTCTCACAgggttccgggcggtgtttc	72nt *gar*
bc1028pilRBS	CTCTGCTCTGACTCTCtttccccctttcaattaggag	bc1028opaeRev	CTCTGCTCTGACTCTCgggttccgggcggtgtttc	32nt *gar*
bc1049pilRBS	ACACGTGTGCTCTCTCtttccccctttcaattaggag	bc1049opaeRev	ACACGTGTGCTCTCTCgggttccgggcggtgtttc	FA1090 *garP*$_{-35}$
bc1006pilRBS	CATATATATCAGCTGTtttccccctttcaattaggag	bc1006opaeRev	CATATATATCAGCTGTgggttccgggcggtgtttc	FA1090 *garP*$_{-10}$
bc1009pilRBS	ACACACGCGAGACAGAtttccccctttcaattaggag	bc1009opaeRev	ACACACGCGAGACAGAgggttccgggcggtgtttc	FA1090
bc1015pilRBS	CGCATGACACGTGTGTtttccccctttcaattaggag	bc1015opaeRev	CGCATGACACGTGTGTgggttccgggcggtgtttc	*rnhA*+*nicsP*$_{lac}$::*rnhA* 0.005 mm IPTG
bc1016pilRBS	CATAGAGAGATAGTATtttccccctttcaattaggag	bc1016opaeRev	CATAGAGAGATAGTATgggttccgggcggtgtttc	*rnhA*+*nicsP*$_{lac}$::*rnhA* 1 mm IPTG
bc1018pilRBS	TCACGTGCTCACTGTGtttccccctttcaattaggag	bc1018opaeRev	TCACGTGCTCACTGTGgggttccgggcggtgtttc	*rnhA*+*nicsP*$_{lac}$::*rnhA* 0 mm IPTG
bc1019pilRBS	ACACACTCTATCAGATtttccccctttcaattaggag	bc1019opaeRev	ACACACTCTATCAGATgggttccgggcggtgtttc	*rnhA+nicsPlac::rnhA garP*$_{-10}$ 0 IPTG
bc1020pilRBS	CACGACACGACGATGTtttccccctttcaattaggag	bc1020opaeRev	CACGACACGACGATGTgggttccgggcggtgtttc	FA1090 *garP*$_{-35}$
bc1032pilRBS	GAGACTAGAGATAGTGtttccccctttcaattaggag	bc1032opaeRev	GAGACTAGAGATAGTGgggttccgggcggtgtttc	72nt *gar*
bc1033pilRBS	TCTCGTCGCAGTCTCTtttccccctttcaattaggag	bc1033opaeRev	TCTCGTCGCAGTCTCTgggttccgggcggtgtttc	32nt *gar*
bc1040pilRBS	TGTCATATGAGAGTGTtttccccctttcaattaggag	bc1040opaeRev	TGTCATATGAGAGTGTgggttccgggcggtgtttc	FA1090 G4 mutant

Supplemental Table 5. Primers used for PacBio Sequencing

Primers were used to amplify the *pilE* gene to test antigenic variation frequencies and different barcodes were used for each strain as listed in the table.