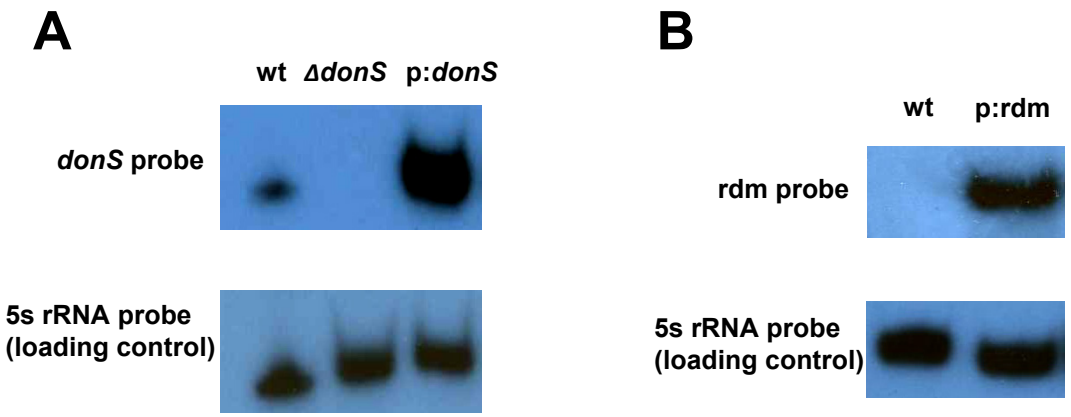
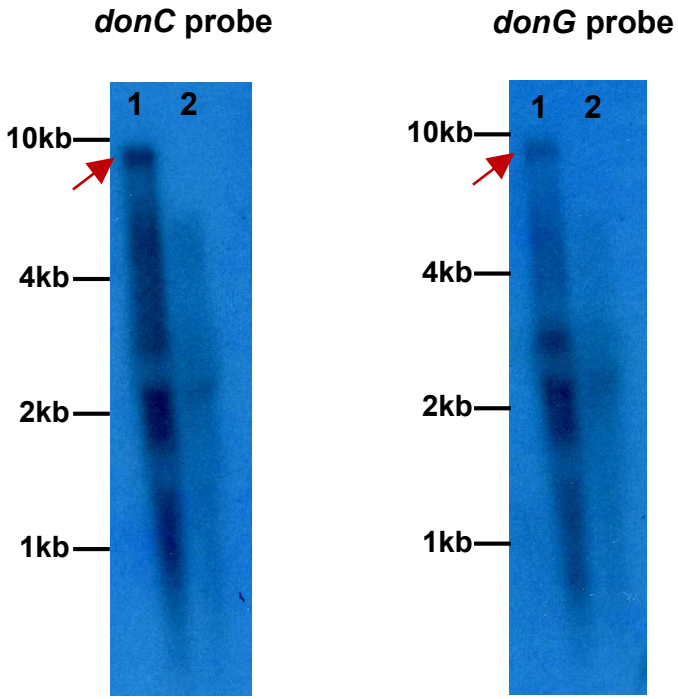
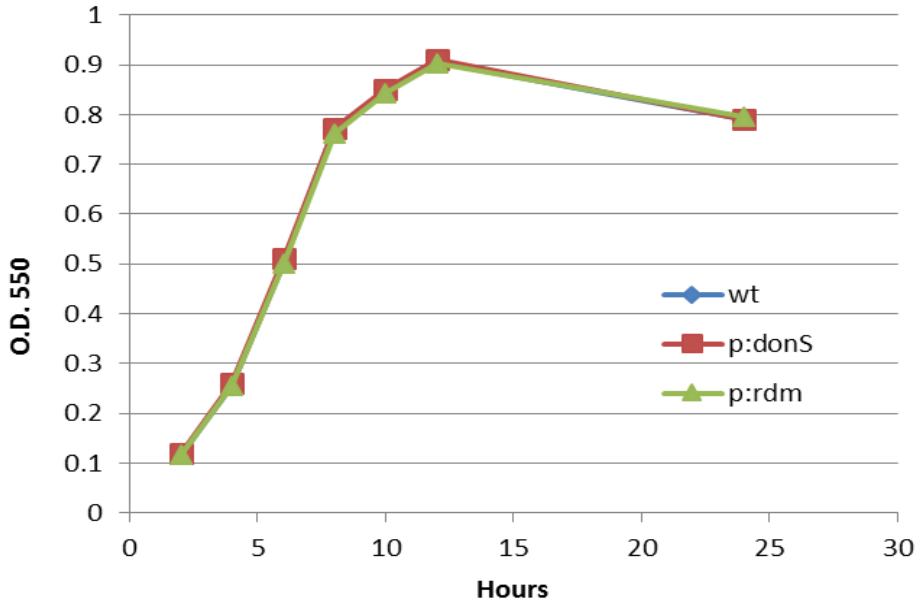


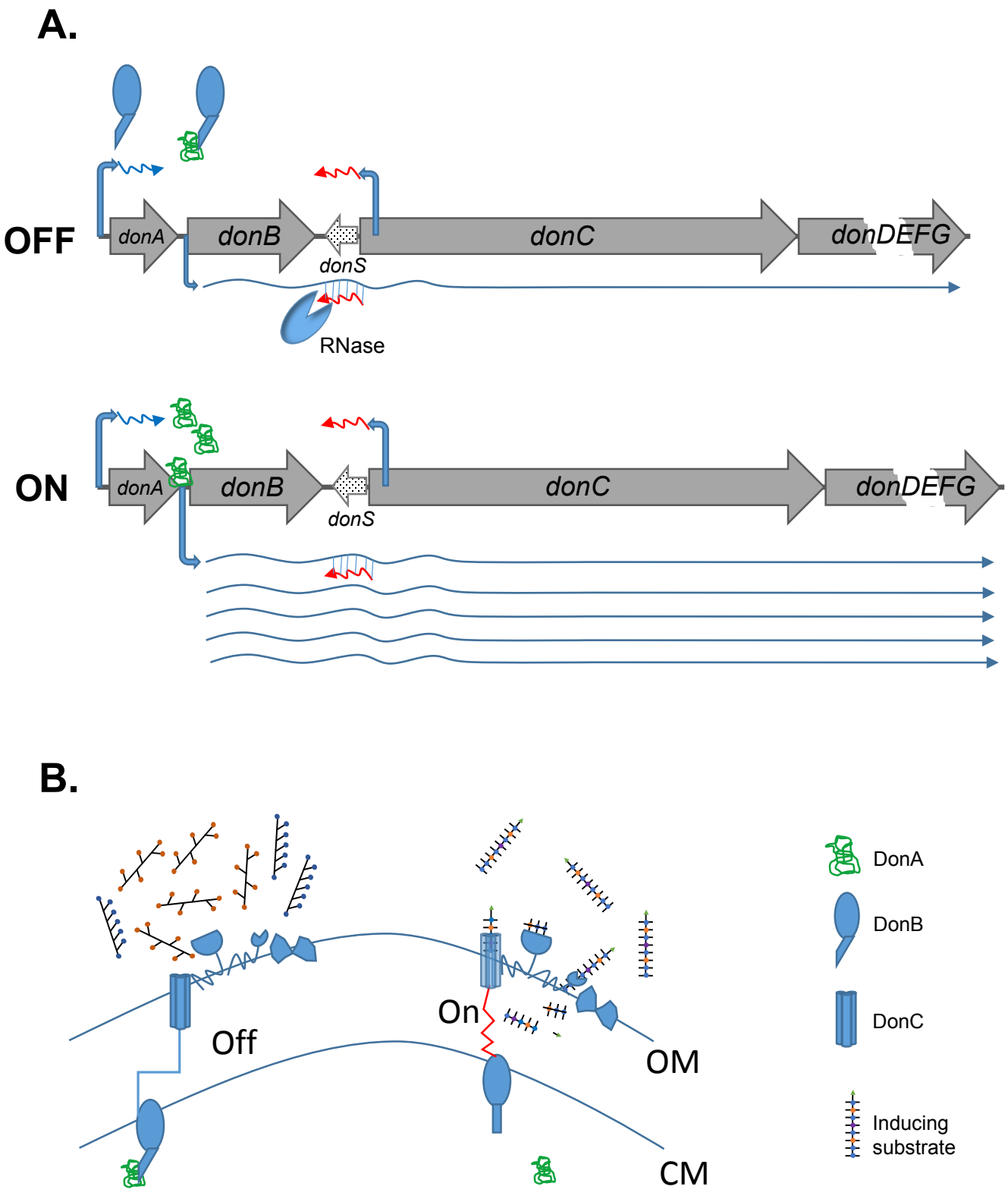
Supplementary Figure 1. Verification of sRNA mutants and constructs. Autoradiographs showing sRNA expression in *B. fragilis* 638R strains. **A.** DonS expression in the wild type strain (wt), *donS* null mutant (Δ*donS*), *donS* overexpression strain, pUC2:*donS (*p:*donS*). **B.** Overexpression of the random sRNA (p:rdm). For each autoradiograph 10 µg of total RNA was analyzed by northern hybridization using γ-^{32}P labeled probes for each sRNA. The 5s rRNA was used as a loading control.



Supplementary Figure 2. Northern analysis of *don* expression when *donS* is overexpressed. Autoradiographs of *donBCDEFG* expression in the *B. fragilis* wild type strain 638R (Lane 1), and *donS* overexpression strain, pYC2:*donS (*Lane 2). 30 µg of total RNA was analyzed by northern hybridization using α-^{32}P labeled probes for detection of *donC* or *donG* mRNA. 16s rRNA was used as a loading control. The full length transcript for the *donBCDEFG* operon is predicted to be 9.5 kb (arrowhead). The full length mRNA is subject to degradation as seen by the diffuse smear of RNA below the intact message. Also note the compression artifacts caused by the high concentration of 16S and 23S rRNA.



Supplementary Figure 3. Growth curves for the wild type strain, 638R (wt), *donS* overexpression strain pYC2:*donS* (p:donS) and random sRNA overexpression strain pYC3:rdm (p:rdm) grown in DM-glucose media. An overnight inoculum of 2% was used and the OD A_{550} was measured at specific time intervals. All experiments represent two biological repeat. Error bars are standard deviation.


A.
OFF
donA
donB
donS
donC
donDEFG
RNase
ON
donA
donB
donS
donC
donDEFG
B.
Off
On
OM
CM
DonA
DonB
DonC
Inducing substrate

Supplementary Figure 4. Model of Don Regulation. A) Transcriptional organization of the Don locus with and without induction. B) The Don protein complex in the absence and presence of inducing substrate.

1. Don OFF: In the absence of inducing substrate the sigma factor DonA (σ^{DonA}) is sequestered by the antisigma factor DonB. A low level of *donBCDEFG* transcript is produced (from a consensus house keeping sigma factor promoter upstream of *donB*). DonS is constitutively transcribed at a low to moderate level during logarithmic phase growth. The levels of DonS likely will exceed the low levels of the *donBCDEFG* message. This will favor base pairing of DonS and the *donBCDEFG* message which will prevent translation and eventually target the RNA complex for degradation. It is expected that there is a very low rate of *donCDEFG* translation that is required for surveillance of the environment for substrate.

2. Don ON: In the presence of inducing substrate, transport of substrate through DonC causes a conformational change in DonB which releases σ^{DonA} and results in a large induction of *donBCDEF*G mRNA such that it greatly exceeds the level of DonS. The ratio of DonS to *donBCDEFG* mRNA remains low in the presence of substrate and this allows for significant translation and amplification of the Don PUL protein complex. In addition it is equally likely that the levels of DonS are kept low by the very act of transcription of the *donBCDEFG* operon. That is, the extremely high rate of *donBCDEFG* transcription driven by σ^{DonA} may cause transcriptional interference of *donS* via a mechanism of collision of opposing RNA polymerases.

3. Don repression: As the concentration of inducing substrate decreases, DonC remains inactive for longer periods of time allowing DonB to revert back to its repressing conformation and sequester σ^{DonA}. As the ratio of DonS/*donBCDEFG* increases there is a rapid decline in the production of the Don PUL such that it will quickly reach an equilibrium with the concentration of substrate.

Supplemental Table 1. Oligonucleotides used in this study[a].

Name	Sequence (5' → 3')	Description
sigOK+2kL	AGTC GGATCC CCGATGGTTACATCTACGATC (BamHI)	Designed to amplify a 1283 bp upstream fragment of *donA* gene
sigOK+2kR	AGTC CTGCAG CGCTTTGACTTTGGGATAAGTC (PstI)	
sigOK-2kL	AGTC CTGCAG CACATCTATTTGGCACTAATCG (PstI)	Designed to amplify a 1231 bp downstream fragment of *donA* gene
sigOK-2kR	GCAT AAGCTT TAACGCAAAGAATTC	
Omp117rtL	GGTGAAGGCATTTCCGACTT	Designed to amplify a 140 bp fragment of *donC* gene for quantitative PCR
Omp117rtR	TTGCCTTCCTGCCCTTTCTT	
16srL	GATGCGTTCCATTAGGTTGTTG	Designed to amplify a 127 bp fragment of 16s ribosomal RNA gene for quantitative PCR
16srR	CACTGCTGCCTCCCGTAG	
anti-sigOK+2kL	CACG AAGCTT GCGTACAGTA (HindIII)	Designed to amplify a 1393 bp upstream fragment of *donB* gene
anti-sigOK+2kR	AGTC CTGCAG GGCAAACAGACGGATGATTC (PstI)	
anti-sigOK-2kL	AGTC CTGCAG GTTGTGGGAGGATTCAGTCAT (PstI)	Designed to amplify a 1296 bp downstream fragment of *donB* gene
anti-sigOK-2kR	AGTC GGATCC CTCCGTATTGTTGGAGATCGA (BamHI)	
sigOK-340L	AGTC GGATCC AAATAAGAAACAATT ATGATTTTAAATAACGAGTCTAATAAGAA G (BamHI)	Designed to amplify the *donA* gene with the *ahpC* gene ribosomal binding site upstream
sigOK-340R	AGTC GAGCTC AGGTGTGACTGATTACTCAA (SacI)	
sRNA117+L	AGTC GGATCC GTCAGCCATTCATTGTCAGA (BamHI)	Designed to amplify a 693 bp DNA fragment including *donS* sequence with its -7 "TTTG" change to "AAAC"
sRNA117+R (AAACmut)	AAATAGACTTTAATCGATAAAAATTCATA GAAAACAACGAATTTTAGTGTTAAATCAT AATATTTATTTCC	
sRNA117-L (AAACmut)	GGAAATAAATATTATGATTTAACACTAAA ATTCGTTGTTT TCTATGAATTTTTATCGATTAAAGTCTATT T	Designed to amplify a 679 bp DNA fragment including *donS* sequence with its -7 "TTTG" change to "AAAC
sRNA117-R	AGTC GGATCC CTGCTGAACACTGTAACTCA (BamHI)	
sRNA117-L	TTGTCTCTTATCTCCTAATGCCTTACTTTT GCATCCCGAATTTTAGTGTTAAATCATAA TATTTATTTC	Designed to amplify the *donS* sequence with an 82 bp 16s rRNA promoter fragment
sRNA117-R1	AGTC GAGCTC GGATTCAGTCATGAACTGAAG (SacI)	

16sPR-36-82	AGTCCTGCAGTTTACGTTTTTATTCAAAA TATTTTCAAAAAAATCCCCTTTTATATTTG TCTCTTATCTCCTAATGCC (PstI)	sequence tagged upstream.
DonSP	GCCACCTGATTCCGGATATGAAATCTGA AT	Probe for small RNA DonS
0549P	GGTGGCACACTTCCGGCTCCGGGTTAAC AA	Probe for small RNA sr0549
1041P	CCTTGTGAGCTTGTTTGGCGACAAGCTG AGA	Probe for small RNA sr1041
1270P	GGTCTGGTACACCTTTCCCTCCCGATAA AGTCAA	Probe for small RNA sr1270
1469P	TGAGAATGCCGATAGGTGTTCCCGCACC TA	Probe for small RNA sr1469
1035P	ATTGGCGTATCTACCTGTATGAAAGTCCA CCGTC	Probe for small RNA sr1035
2917P	ACATATTGGGGTATGTCCTCCGATTCAG AA	Probe for small RNA sr2917
3123P	CACCCATTTCCGATCCCCGAAATCAATC AA	Probe for small RNA sr3123
3597P	CTGAGTTTTTTTAATCACGTCTCTTGCAG GAGGCGC	Probe for small RNA sr3579
3604P	CCTCATATCATCCAACCCCTGATTAGATT AAACTACG	Probe for small RNA sr3604
4145P	CAGATGCGCCAACACCTGCCAACCTCGG GT	Probe for small RNA sr4145
3787P	AGACTTTGGACGGAATATACCCCCTCGC CCTACGCCTT	Probe for small RNA sr3787
3799P	GGTACTGCAATACCTGCCAGCTAAAAGG AGAAACATTCTTCTTTTCCC	Probe for small RNA sr3799
3821P	GGGTGAGCGCTACCAACACTCACCCCCA AAAG	Probe for small RNA sr3821
5srRNAP	CACTGTTACGCAGTACCATCGGCGTGAT CA	Probe for the 5s ribosomal RNA
DonS-L	AGT ATC CGG AAA GAG GCG G	qRT-PCR primer pair to measure the sRNA DonS
DonS-R	CCC AAA AAG TAA AGC ATA AGC TTT AAC G	
Sr1035L	ACAGGAAGATATTGGCGTATCT	qRT-PCR primer pair to measure the donS –like sRNA sr1035
Sr1035R	ATT CTA TTT GCC GCA CAG TTT GT	
Sr1041L	TGG AAA GAT ATT GGC GTA TCT AAT CC	qRT-PCR primer pair to measure the donS –like sRNA sr1041
Sr1041R	TTTATCTCAGCTTGTCGCCAAAC	
Sr1270L	AGG AAA TGG TCT GGT ACA	qRT-PCR primer pair to measure the donS –like sRNA sr1270
Sr1270R	TAATAAGGTTAATACTTTGTTGTTTTT TTATTTGGCTC	
Sr1496L	AAG TTA ACC TAT TGA GAA TGC	qRT-PCR primer pair to

	CGA T	measure the donS –like sRNA sr1496
Sr1496R	ATA AAA AAT TGT GTT GAT TTC TTA TGC C	
Sr3123L	CAATTAATACTGTTTAAAGTCTCTAAA GG	qRT-PCR primer pair to measure the donS –like sRNA sr3123
Sr3123R	GAA CCG GAA ACA AAT TAC CAC A	
Sr3597L	AAA GTA AAT TGC GCC TCC TGC	qRT-PCR primer pair to measure the donS –like sRNA sr3597
Sr3597R	ATA GGT AAA TGT TGC AGC ACT TAC C	
Sr3787L	AAA TTT AAG GCG TAG GGC GAG	qRT-PCR primer pair to measure the donS –like sRNA sr3787
Sr3787R	AAA AGT AGG GAA GGG GAA ATA GAC T	
Sr3821L	GTG AGC GCT ACC AAC ACT CA	qRT-PCR primer pair to measure the donS –like sRNA sr3821
Sr3821R	TAC TAT AGG TCT GTT TCG AGA TGG A	
Sr4145L	TCT GAA TTT TTG AAA GGG TTT GGA ATA CG	qRT-PCR primer pair to measure the donS –like sRNA sr4145
Sr4145R	TCG GCA GAT GCG CCA ACA	
donC-L-NB	CGT AGG CTA TAC CAC CCA ATA	Designed to amplify a 328 bp fragment of *donC* gene as northern hybridization probe
donC-R-NB	GTC CAC TAC GTA GAG CAC ATT	
donG-L-NB	CGA GAA CTG CAC CTT TGA A	Designed to amplify a 330 bp fragment of *donG* gene as northern hybridization probe
donG-N-NB	TTG GCT GTG AAG TTG CCA T	

[a] Restriction site sequences if present are underlined.

Supplemental Table 2. Construction and sequence of the random sRNA molecule (rdm).

Designation	**Sequence**
rdm sequence [a]	CAGGTAGATTATGTAAGTTGAGAGATGCAGGAAAAGTTCTTAACC TTCTCATAGGACGTGAACTTATTCTCTAATAGAGCTGGTTCATCCC TTTCGGTCGAAAGACCGAAGGGGATGACTTGTTTTATGCC
	Primers
Rdm-L	CAGGTAGATTATGTAAGTTGAGAGATGCAGGAAAAGTTCTTAACC TTCTCATAGGACGTGAACTTATTCTCTAAT
Rdm-R	GGCATAAAACAAGTCATCCCCTTCGGTCTTTCGACCGAAAGG GATGAACCAGCTCTATTAGAGAATAAGTTCACGTCC
Rdm-L-L	**TTGTCTCTTATCTCCTAATGCC**TTACTTTTGCATCCCAGGTAGATT ATGTAAGTTGAGAGA
Rdm-R-R	AGTC GAGCTC GGCATAAAACAAGTCATCCCCTTCGGTCTTTCG (SacI)
16sPR-36-82	AGTC CTGCAG TTTACGTTTTTATTCAAAATATTTTCAAAAAAATCCCCTTTTATAT**TT GTCTCTTATCTCCTAATGCC** (PstI)
Construction Strategy	
Oligonucleotide primers used in the synthesis of random sRNA (rdm) are listed above. Rdm-L and Rdm-R have a complementary region shown by the blue text. To construct the rdm sequence the Rdm-L and Rdm-R primers were incubated together in the thermal cycler for 30 cycles and the PCR product was extracted from an agarose gel. Using this PCR product as a template, a second round of PCR was	

performed with the primer pair Rdm-L-L/Rdm-R-R. These primers added a SacI restriction site at the 3' end of the sequence and they added about half of the 16S rRNA promoter region to the 5' end of the molecule. This PCR product was extracted from an agarose gel and used in a third PCR reaction with the primer pair 16sPR-36-82/Rdm-R-R. The primer 16sPR-36-82 overlapped with a portion of the Rdm-L-L primer (shown in orange text).The primer 16sPR-36-/82 added the remainder of the 16S rRNA promoter and a PstI restriction site. This final PCR product was extracted from an agarose gel and cloned it into pFD340 between the PstI and SacI sites.

[a] Random sRNA sequence shown with the black and blue text and the 5s rRNA terminator region is in red text.

Supplemental Table 4. Properties of glycan hydrolases in the *B. fragilis* sRNA-associated PULs

sRNA Designation	Approx. Size (nucleotides)	Cognate *susC* homolog	Hydrolase and binding motifs associated with the PUL gene products	Potential substrates for PUL
DonS	128	*donC* (*BF638R_3439*)	Endo-β-N-acetylglucosaminidases (GH18); Concanavalin A/LamG binding motif	Complex N-linked serum glycoproteins (confirmed)
sr0549	100	*BF638R_0549*	α-glucosidase (GH31); α-galactosidase (GH36)	Glycoproteins, O-glycans
sr1035	120	*BF638R_1035*	EEP domain nuclease/phosphatase; MPP family metalophophatase/hydrolase	Nucleic acids,
sr1041	121	*BF638R_1041*	AslA family arylsulfatase; AslA family arylsulfatase	Glycosaminoglycans
sr1270	108	*BF638R_1270*	MPP_DCR2 family metalophosphatase; Alkaline phosphatase family (pyrophosphatase)	Nucleic acids, phosphoproteins
sr1496	102	*BF638R_1496*	α-galactosidase (GH27)	Glycoproteins
sr2917	78	*BF638R_2917*	β-glucosidase (GH3); CmuC-like corrinoid methyltransferase; uroporphyrinogen decarboxylase; corrinoid methyltransferase; methionine synthase activation domain; β-glucosidase (GH3)	
sr3123	109	*BF638R_3123*	Chitobiase (GH20); AslA family arylsulfatase	N-Glycans, glycosaminoglycans
sr3597	99	*BF638R_3597*	MPP metalophosphatase (partial) + pyrrolo-quinolone quinone (PQQ) domain; phosphodiesterase/pyrophosphatase Npp1 family; EEP-2 family exoDNase III	Nucleic acids
srCcf	99	*ccfC* (*BF638R_3604*)	DUF 1735 (acylhydrolase associated); discoidin (factor V and VII) binding family, α-n-acetylglucosaminidase	Phospholipid binding, glycolipids
sr3787	98	*BF638R_3787*	Sialidase/neuraminidase; Sialidase/neuraminidase; MFS family carbohydrate transporter	Glycoproteins, gangliosides
sr3799	100	*BF638R_3799*	AslA family arylsulfatase; AslA family arylsulfatase	Glycosaminoglycan
sr3821	112	*BF638R_3821*	Xylanase domain (partial); Concanavalin A/LamG binding motif + EEP nuclease/phosphatase;	Nucleic acids
sr4145	99	*BF638R_4145*	Exo-β-glucosaminidase or galactosidase (GH35)	Hybrid N-glycans, glycosaminoglycans