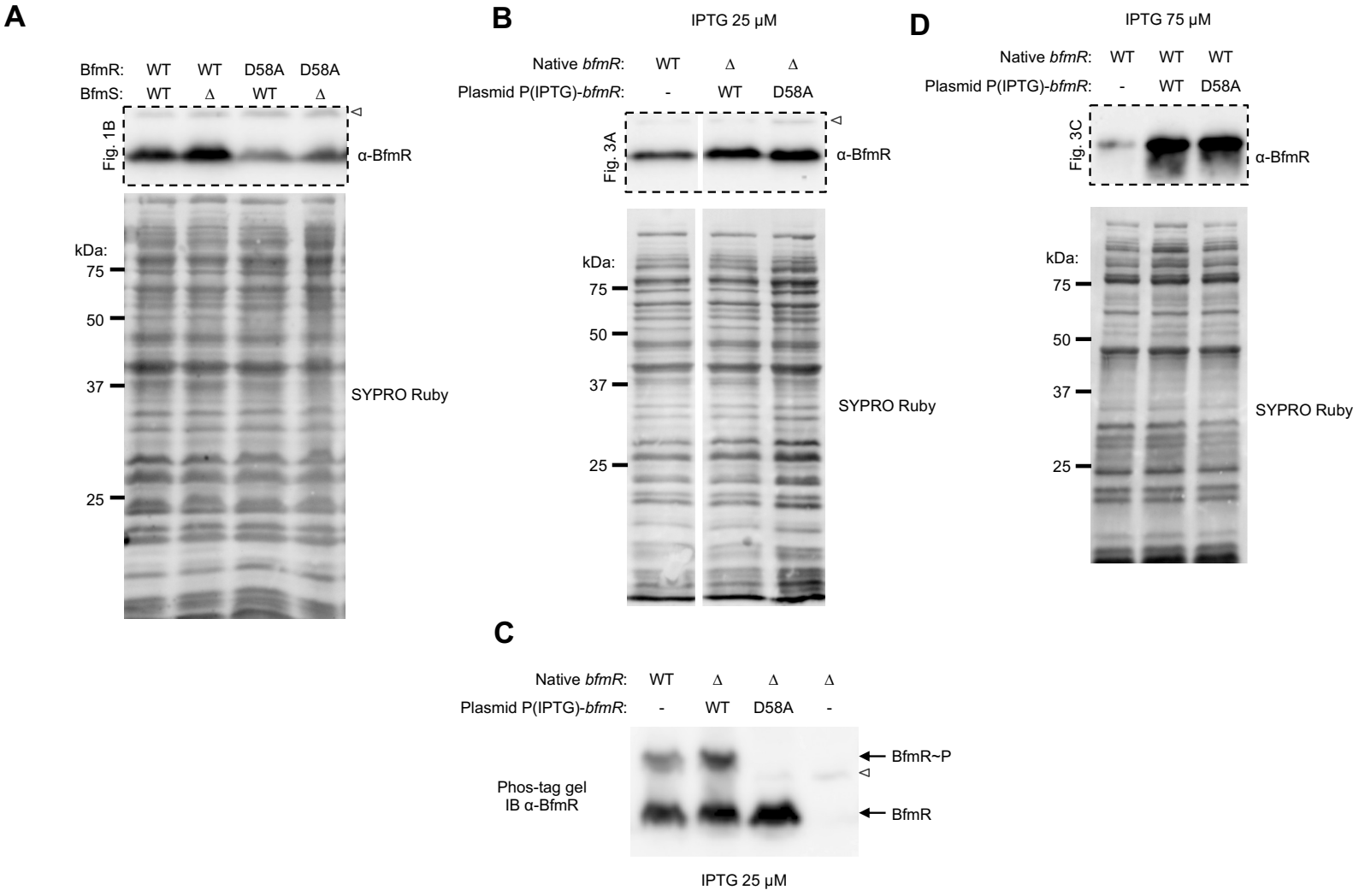

Fig. S1. Supplemental information from Western blots. (A, B and D) SYPRO staining of total protein in the blotted samples from Fig. 1B, 3A, and 3C, respectively. kDa values indicate the migration of molecular weight markers. (C) Phos-tag immunoblot of samples from Fig. 3A. Open arrowheads indicate non-specific protein band reacting weakly with BfmR antiserum.

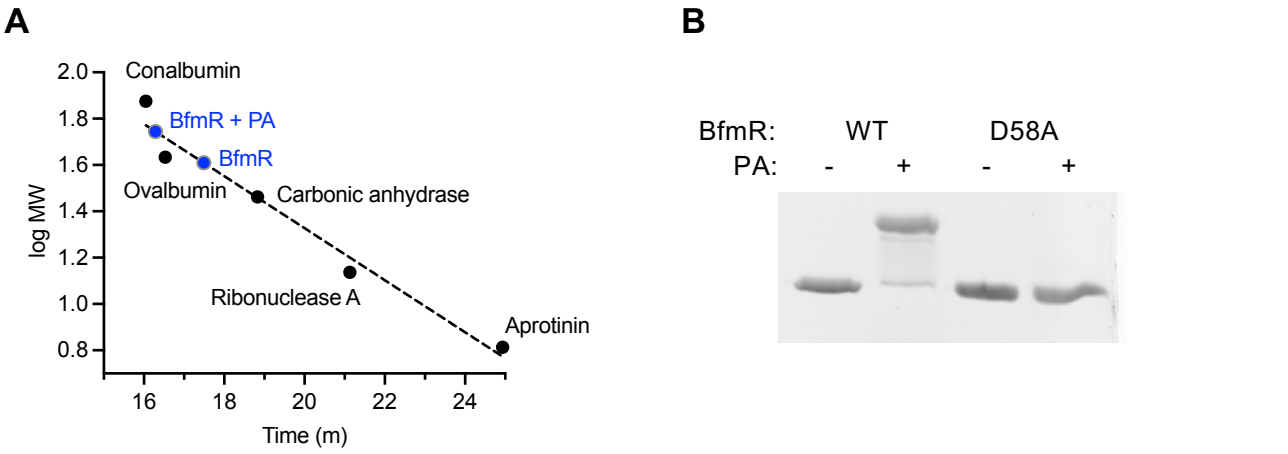

Fig. S2. Analyses of BfmR *in vitro*. (A) Estimation of BfmR molecular weight relative to protein standards without and with phosphorylation (Buffer S). Retention times of the protein standards (Gel Filtration LMW Calibration Kit, Cytiva) was plotted vs molecular weight. Estimated size of BfmR and BfmR+PA was determined by plotting their average peak retention time (n=3). (B) Efficient phosphorylation of purified BfmR protein used in MST experiments. BfmR was treated with or without PA in Buffer D, and phosphorylation analyzed by Phos-tag gel as in Fig. 4.

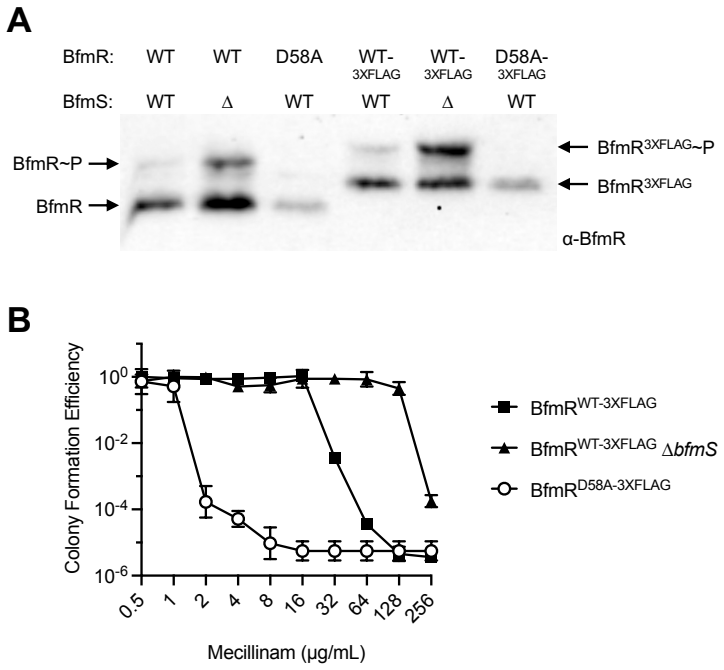

Fig. S3. C-terminal 3XFLAG epitope tag preserves BfmR functions. (A) Phos-tag Western blot analysis of BfmR phosphorylation in strains with the indicated *bfmRS* alleles. Blots were probed with BfmR antiserum. (B) Strains containing a C-terminal 3XFLAG tag on BfmR show levels of resistance to mecillinam resistance consistent with that seen with untagged strains [4]. Colony forming efficiency was measured on solid medium with mecillinam at the indicated dose compared to control medium lacking drug. Data points show geometric mean ± s.d. (n=3).

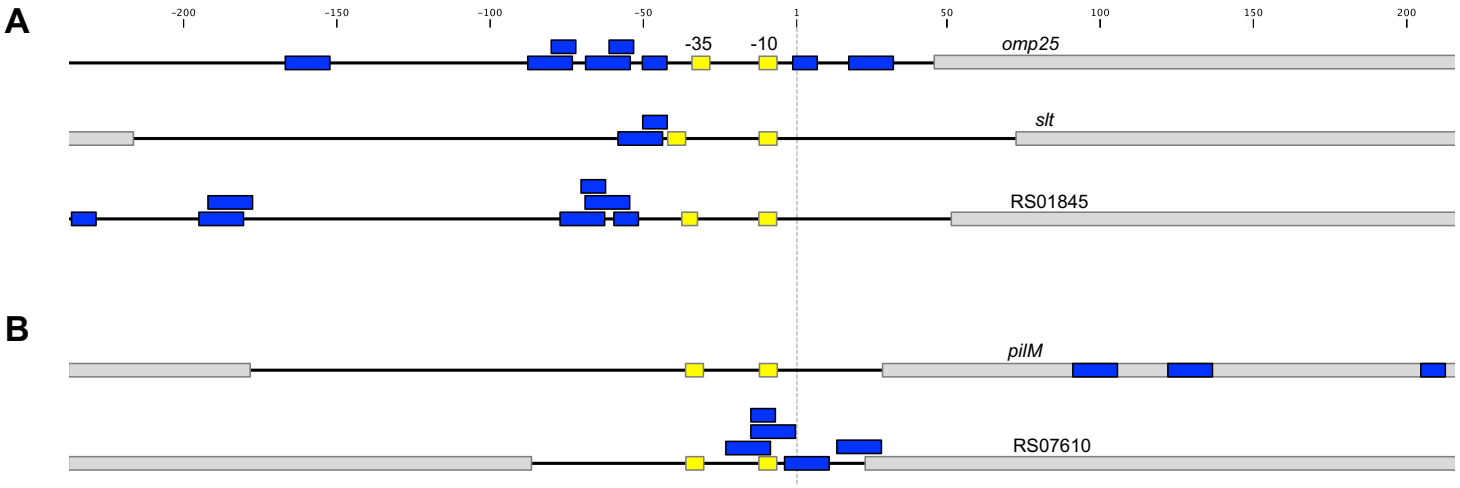

Fig. S4. Locations of BfmR binding motifs relative to promoters of example direct target genes. Promoter regions of activated (A) and repressed (B) targets are shown. Location of promoter elements was determined from [10] (*slt*, RS01845), [11] (*omp25*), or predicted by using BPROM software [12] (*pilM*, RS07610). -35 and -10 elements are depicted as yellow boxes. 15bp motif and 8bp BfmR binding motifs are shown as wide and narrow blue rectangles, respectively. ORFs are shown as grey rectangles. Dotted vertical line indicates the location of the TSS.

Candidate target sRNA			ChIP-seq		
ID	Name	Locus coordinates	Closest summit	Distance from sRNA	FE
sRNA21	Aar	833848 - 833746	833899	-51	10.39
sRNA84	6S/SsrS	2772408 - 2772589	2772420	12	3.91
sRNA77		2544991 - 2544831	2544991	0	7.11
sRNA103		3560294 - 3560386	3560284	-10	5.91
sRNA40		1714402 - 1714497	1714456	54	3.8
sRNA29		1381844 - 1381668	1382030	-186	2.77
sRNA54		2180417 - 2180273	2180601	-184	2.86
sRNA76		2544740 - 2544594	2544991	-251	7.11
sRNA87		2841274 - 2841173	2841524	-250	2.25
sRNA30		1396374 - 1396483	1396224	-150	5.14
sRNA85		2774620 - 2774564	2774684	-64	4.09

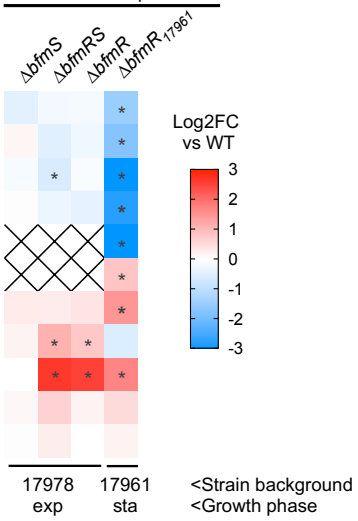

Fig S5: Analysis of sRNAs that are candidates for direct regulation by BfmR. sRNA loci were identified that are in proximity to a BfmR binding site identified by ChIP-seq. Locus coordinates indicate the position of the sRNA gene in 17978 (NZ_CP012004). FE indicates ChIP-seq fold enrichment in NRA49 (Δ*bfmS*). Heatmap shows log2 fold change in RNA-seq reads, comparing the indicated mutant to WT. X indicates raw read count was too low to analyze. *, adjusted p-value <0.05; exp, exponential phase; sta, onset of stationary phase. Gray shading indicates candidates not showing significant BfmRS-dependent change in gene expression in RNAseq.

Table S1. Strains, plasmids, and primers used in this study

Strains

Designation	Genotype or description	Strain ID	Reference
A. baumannii			
17978	cerebrospinal fluid isolate, AbaAL44^{+} ("UN") type, ATCC	EGA83	[1, 2]
17978 Δ*bfmS*	ATCC 17978 Δ*bfmS*::*aacC1*	EGA195	[3]
17978 Δ*bfmRS*	ATCC 17978 Δ*bfmRS*::*aacC1*	EGA495	[4]
17978 Δ*bfmR*	17978 Δ*bfmR*::*aacC1*	EGA496	[4]
17978 *bfmR*(D58A)	17978 *bfmR*(D58A)	NRA407	This work
17978 *bfmR*(D58A) Δ*bfmS*	17978 *bfmR*(D58A) Δ*bfmS*	NRA446	This work
17978 *slt*p-GFP	EGA83 with pEGE315	NRA480	This work
17978 *bfmR*(D58A) *slt*p-GFP	NRA407 with pEGE315	NRA481	This work
17978 Δ*bfmRS slt*p-GFP	EGA495 with pEGE315	NRA482	This work
17978 *pilM*p-GFP	EGA83 with pNRE216	NRA433	This work
17978 Δ*bfmS pilM*p-GFP	EGA195 with pNRE216	NRA435	This work
17978 *bfmR*(D58A) *pilM*p-GFP	NRA407 with pNRE216	NRA437	This work
17978 Δ*bfmR* P(IPTG)-*bfmR*	EGA496 with pJE86	NRA460	This work
17978 Δ*bfmR* P(IPTG)-*bfmR*(D58A)	EGA496 with pNRE138	NRA461	This work
17978 Δ*bfmR* vector	EGA496 with pEGE305	NRA462	This work
17978 P(IPTG)-*bfmR*	EGA83 with pJE86	NRA147	This work
17978 P(IPTG)-*bfmR*(D58A)	EGA83 with pNRE138	NRA456	This work
17978 vector	EGA83 with pEGE305	NRA365	This work
17978 *bfmR*-3xFLAG	17978 *bfmR*-3xFLAG	NRA28	This work
17978 *bfmR*(D58A)-3xFLAG	17978 *bfmR*(D58A)-3xFLAG	NRA29	This work
17978 *bfmR*-3xFLAG ΔbfmS	17978 *bfmR*-3xFLAG Δ*bfmS*	NRA49	This work
17978 Δ*gtr6*	17978 Δ*gtr6*	JBA202	[5]
17978 Δ*gtr6 bfmR*(D58A)	17978 Δ*gtr6* bfmR(D58A)	NRA486	This work
E. coli			
DH5α	*supE44* Δ*lacU169* (ϕ80lacZΔM15) *hsdR17 recA1 endA1 gyrA96 thi*-1 *relA1*	EGE1	[6]
DH5λpir	DH5α (λpir) *tet*::Mu *recA*	EGE4	[7]
BL21 (DE3) pLysS	F- *ompT hsdSB*(rB- mB-) *gal dcm* (DE3) pLysS (Cm^{r})	NRE81	Novagen

Plasmids

Plasmid	Description	Reference
pUC18	*oriColE1* MCS Cb^{r}	[8]
pEGE305	P(IPTG) shuttle vector (*ori*-*pBR322 ori*-pWH1277 *bla*::*lacI*^{q}-T5*lac*P Tc^{r})	[4]
pJB4648	Conditionally replicating allele exchange plasmid (*oriTRP4 oriR6K sacB* Gm^{r})	[9]
pNRE157	pUC18 containing homology arms for introducing *bfmR*(D58A) allele	This work
pNRE169	pJB4648 containing homology arms for introducing *bfmR*(D58A) allele	This work
pEGE245	reporter plasmid with promoterless *gfp*mut3 (*ori*-pBR322 *ori*-pWH1277, Tc^{r})	[4]
pEGE315	*slt*p-GFP reporter plasmid (*ori*-pBR322 *ori*-pWH1277, Tc^{r})	[4]
pNRE211	pUC18::*pilM*p	This work
pNRE216	pEGE245::*pilM*p-GFP	This work

Table S1 (continued)

pJE83	pUC18::*bfmR*	This work
pJE86	pEGE305::*bfmR*	This work
pNRE137	pUC18::*bfmR*(D58A)	This work
pNRE138	pEGE305::*bfmR*(D58A)	This work
pNRE80	pUC18::[NdeI]*bfmR*[BamHI]	This work
pNRE85	pET28a::*bfmR*	This work
pNRE127	pET28a::*bfmR*(D58A)	This work
pEGE228	pUC18 containing *bfmR*-3xFLAG-*bfmS* allelic exchange construct	This work
pNRE26	pJB4648 containing *bfmR*-3xFLAG-*bfmS* allelic exchange construct	This work
pNRE12	pUC18 containing *bfmR*(D58A)-3xFLAG-bfmS allelic exchange construct	This work
pNRE27	pJB4648 containing *bfmR*(D58A)-3xFLAG-bfmS allelic exchange construct	This work
pNRE31	pUC18 containing upstream homology arm for *bfmS* deletion with *bfmR*-3xFLAG	This work
pNRE32	pUC18 containing upstream homology arm for *bfmS* deletion with *bfmR*(D58A)-3xFLAG	This work
pNRE33	pUC18 containing downstream homology arm for *bfmS* deletion	This work
pNRE34	pJB4648 containing homology arms for Δ*bfmS* deletion with bfmR-3xFLAG	This work

Oligonucleotide primers

Primer name	**Sequence (5' – 3'; restriction site underlined if present)**	**RE site(s)**
Allelic exchange		
bfmR-D58x-R	AGACCACAAGATCCGGTTGCTC	
bfmR-D58A-F	TGGCTGTCATGTTGCCGGGTGC	
dbfmS-down-F	CATGTCGACGCAATTGCCCATGATGAACT	SalI
dbfmS-down-R	CATGGTACCTTTAAACAACCGCCATTAAAGACC	KpnI
dbfmS-up-F	TATGGTACCACTGTGTTTAAACACTCGACCAACC	KpnI
dbfmS-up-R	ATCGGATCCAGTTTGGTGAACGCCTACTTGT	BamHI
BamHI-500up-BfmRD58A-F	ACATGGATCCCGGTAGATCAATCTTGACTTT	BamHI
SalI-500dwn-BfmRD58A-R	ATGTGTCGACGATTTTACAATCCATTGGTTTCTTTAAC	SalI
Reporter/bfmR expression		
SacI-pilMp-Fwd	ATGTGAGCTCGATCAAAAAAATTGGACGCACG	SacI
KpnI-pilMp-Rev	TAGCGGTACCACTATTGTCCTATTATTTTTTTATCCCC	KpnI
bfmRS-ecoF	GTGGAATTCGCAAATGATAAACGAATGTATCTGCAAG	EcoRI
bfmRonly-pstR	TTACTGCAGCGACCAACCTTATAGGAAGTTTAATCAG	PstI
NdeI-BfmR-Fwd	ACTGCCATATGAGCCAAGAAGAAAAG	NdeI
BamHI-BfmR-Rev	CAGTGGATCCTTACAATCCATTGGTTTCTTTAAC	BamHI
ChIP-qPCR		
dnaA-qF1	GTAGATTCTCGTCCTGGTAGTATTT	
dnaA-qR1	CCTTAGCAGGTTGAGGTATAGG	
bfmR qPCR FWD Set 2	TCGTCGTCTTCAACGATCAGAA	
bfmR qPCR REV Set 2	GCAAATGATAAACGAATGTATCTGCAAG	
surA Set 1F	CTATGCCTATGCGCCATACAA	
surA Set 1R	CATGACCATAGAAGCGGTAAGG	
01845 Set 2F	GTTGTTCATGTGATACATGCCTAT	

Table S1 (continued)

01845 Set 2R	AGCAATTATTATGCCGTTTCCTC	
itrA Set 1F	GACCGTACCAGAAACAGCAT	
itrA Set 1R	TCGCCGCAAAGGTTTACA	
18040 Set 1F	CATCTTCGCGCTGCCTATAA	
18040 Set 1R	ATTGCAGAATTTGTACCGCTATTAC	
wzi Set 1F	ACAGCCGATGAAGCAGTT	
wzi Set 1R	ACTTTGAGAATTGTGCTGACAT	
omp25 Set 1F	AGCACATGGTTACAGTCCAG	
omp25 Set 1R	TGTTACAACTTTCAGTCTAGAGCA	
ompA Set 2F	TCAAGCACTTGGAAAGTCTATCA	
ompA Set 2R	TTGTTGTTCAAGCTCAGCCTA	
pilM/pbp1a Set 1F	GGACAATAGTGTGCTCAGGTTAT	
pilM/pbp1a Set 1R	CTTGACAGAGAGCTCTAACAACTTA	
gidA Set 1F	TGGCTTACCGTAAATCATGTCA	
gidA Set 1R	CGCCACCGATAACGATAACA	
putP Set 2F	GCGACCTGGATTAGGTTACAA	
putP Set 2R	TGTAGCACGGTAGGCAAATAA	
efp Set 2F	AACCGGGTAAAGGCCAAG	
efp Set 2R	CGCCATCGTTGTATAGGTAGTT	
aar-ChIP qPCR-1F	GGTGATCACTGCGTAGAACAA	
aar-ChIP qPCR-1R	GCGTCACTAATATAACTTGAGTAGGT	
RS07610-ChIP qPCR-2F	CGCTTGGCTAATGTTGTTAGTC	
RS07610-ChIP qPCR-2R	GCTCATTATCTAAATCGACACTTACTC	
sRNA77-ChIP qPCR-4F	GTGGATCGAGGAGATATTACGATTAC	
sRNA77-ChIP qPCR-4R	ACCCAAATGGCGTCGAAA	
RT-qPCR		
rpoC-qF4	CAAACGGTGAGCCAATCATC	
rpoC-qR4	GCCTTCACCTTTCGCATTT	
pilM-qPCR 2F	GCTCTCTGTCAAGAACGGTAAA	
pilM-qPCR 2R	CTGCAACTGCTTCTGGATTTAAG	
slt70-qF1	CACTAGGCCGTTTAGCAAATAAT	
slt70-qR1	GGCTACGGTTCGATAGAGATAC	
aar qPCR FWD Set 1	TGATATGAACCTCACGACATTTCT	
aar qPCR REV Set 1	GGTGATCACTGCGTAGAACAA	
sRNA77-qPCR-1F	ATTTGCTCTTTGCTAGCTGTTT	
sRNA77-qPCR-1R	GGTAATCGTAATATCTCCTCGATCC	
MST		
omp25-FB-FAM	/56-FAM/ATATATTAAATTAAATATAGTTACATAAAAAGCACATGGTTACAGTCCAGTTACTTGGACAAGAT	
omp25-RB	ATCTTGTCCAAGTAACTGGACTGTAACCATGTGCTTTTTATGTAACTATATTTAATTTAATATAT	
adc-FAM-F	/56-FAM/TATTTAAAAAGAAAGATGCCTACTTTTATAACAAAAATCACCTAATTTAATTGTTATGTTTTATA	
adc-R	TATAAAACATAACAATTAAATTAGGTGATTTTTGTTATAAAAGTAGGCATCTTTCTTTTTAAATA	

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