SUPPLEMENTARY INFORMATION

Introduction of the site-directed mutations in the *mrp* operon

For construction of pTFCHS7MrpA-Y136A, pTFCHS7MrpA-E140A, pTFCHS7MrpA-K223A, pTFCHS7MrpA-H230A, pTFCHS7MrpA-H230K, pTFCHS7MrpA-W232A, pTFCHS7MrpA-Y258A, pTFCHS7MrpA-K299A, pTFCHS7MrpA-H345A, pTFCHS7MrpA-G392A, and pTFCHS7MrpA-G392R, pGEMmrp*Eco*RI-*Nsi*I containing part of *mrpA* gene except for the 3' region was constructed. The truncated *mrpA* fragment of *Eco*RI- and *Nsi*I-digested pGEMmrp16-7 was ligated with the *Eco*RI- and *Nsi*I-digested pGEM7zf(+), yielding pGEMmrp*Eco*RI-*Nsi*I. This plasmid was methylated and then used as a template for the mutagenesis system in which PCR was performed with respective sets of primers that contained the target mutations. All primer sequences are provided in Table S2. The PCR products were transformed into *E. coli* DH5α-T1^R. The host cell circularized the linear mutated DNA, and McrBC endonuclease in the host cell digested the methylated template DNA, leaving only unmethylated, mutated product. For the construction of plasmid possessing full length *mrp*, the fragments containing one mutation from *EcoR*I- and *Nsi*I-digested pGEMmrp*Eco*RI-*Nsi*I were ligated with pGEMmrpTFCHS7 digested by *Eco*RI-*Nsi*I.

For construction of pTFCHS7MrpA-F405A, pTFCHS7MrpA-H700A, pTFCHS7MrpA-H700K, pTFCHS7MrpA-H700W, pTFCHS7MrpB-H34A, pTFCHS7MrpB-P37G, pTFCHS7MrpB-F41A, pTFCHS7MrpC-Q70A, pTFCHS7MrpC-T75A, pTFCHS7MrpC-G82I and pTFCHS7MrpC-G82P, pGEMmrpTFCHS7-*Pst*I containing part of the *mrpA* to *mrpC* region was constructed. The *mrpA* to *mrpC* fragments of *Pst*I-digested pGEMmrpTFCHS7 were ligated with the *Pst*I-digested pGEM5zf(+), yielding pGEMmrpTFCHS7-*Pst*I. The subsequent methods were the same as described above. For the construction of plasmid possessing full length *mrp*, the fragments containing one mutation from *Pst*I-digested pGEMmrp*Pst*I were ligated with pGEMmrpTFCHS7 digested by *Pst*I.

For construction of pTFCHS7MrpD-F135A, pTFCHS7MrpD-F136A, pTFCHS7MrpD-F136G, pTFCHS7MrpD-F136E, pTFCHS7MrpD-F136T, pTFCHS7MrpD-E137A, pTFCHS7MrpD-E137D, pTFCHS7MrpD-E137Q, pTFCHS7MrpD-K219A and pTFCHS7MrpD-W228A, pGEMmrp-*Sph*I containing *mrpD* and the downstream region was constructed. pGEMmrpTFCHS7 was digested by *Sph*I and self-ligated in order to remove the *mrpA* to *mrpC* region, yielding pGEMmrp-*Sph*I. The same subsequent methods as described above were then used. For the construction of plasmid possessing full length *mrp,* the *mrpA* to *mrpC* fragments of *Sph*I-digested pGEMmrpTFCHS7 were ligated with the *Sph*I-digested pGEMmrp-*Sph*I containing one mutation.

For construction of pTFCHS7MrpE-T113A, pTFCHS7MrpE-T113Y, pTFCHS7MrpE-P114A and pTFCHS7MrpE-P114G, the strategy was described previously [main text (22)].

For construction of pTFCHS7MrpF-P28G, pTFCHS7MrpF-D32A, pTFCHS7MrpF-R33A, pTFCHS7MrpG-P81G, and pTFCHS7MrpG-P81A, pGEMmrp-*Bam*HI containing the *mrpE* to *mrpG* region was constructed. The *mrpE* to *mrpG* fragment of *Bam*HI-digested pGEMmrpTFCHS7 was ligated with the *Bam*HI-digested pGEM7zf(+), yielding pGEMmrp-*Bam*HI. The same methods described above were then followed. For the construction of plasmid possessing full length *mrp*, each pGEMmrp-*Bam*HI containing one of the mutations was digested by *Bam*HI and ligated with pGEMmrpTFCHS7 digested by *Bam*HI.

Supplementary Figure Legends

Figure S1. Alignment of Mrp subunits of *Bacillus pseudofirmus* OF4 with Mrp subunits from various organisms. The alignment was carried out by the ClustalW program. Amino acid sequence sources are BP, *Bacillus pseudofirmus* OF4, BH, *Bacillus halodurans* C-125, BS, *Bacillus subtilis* 168, SA, *Staphylococcus aureus* (in which Mrp is named Mnh), SM, *Sinorhizobium meliloti* (in which Mrp is named Pha), PA, *Pseudomonas aeruginosa* and VC, *Vibrio cholerae*. Sequence sources of NDH-1 Subunits are EC, *Escherichia coli* and RC, *Rhodobacter capsulatus*. Transmembrane segments were predicted and shown by 3 colors, yellow, green and blue. Con PredII (http://bioinfo.si.hirosaki-u.ac.jp/~ConPred2/), HMMTOP (http://www.emzim.ju/hmmtop/) and TMHMM (http://www.cbs.dtu.dk/services/TMHMM/) were used to predict secondary structure of each Mrp subunit. The stretches of residues in which three methods agreed in their prediction of a transmembrane segment are highlighted in yellow, and areas in which two or fewer methods agreed are in green. The stretches of residues in which HMMTOP and TMHMM, but not Con Pred II methods agreed in their prediction of a transmembrane segment are highlighted in blue. Amino acid residues indicated by a red “Na” have been proposed to have roles in Na^+ binding [main text (38)]. Histidine residues indicated by a red “Q” are in possible quinone-binding sites [main text (35-37)]. Mutations of glycine residues indicated by a red “C-125” have been previously reported to lead to alkali-sensitivity in *B. halodurans* C-125 [main text (11)].

Figure S2. Effect of mutations on the capacity of Mrp systems to complement the sodium-sensitivity of *E. coli* KNabc. *E. coli* KNabc transformants were grown at 37 ℃ for 16 h in LBK medium, at pH 7.5, containing added NaCl at the indicated concentrations. The A_{600} of the cultures was then measured. The error bars indicate standard deviations for the results from duplicate cultures in three independent experiments.

Figure S3. Na$^+$/H$^+$ antiport activity in everted membrane vesicles from *E. coli* KNabc transformants expressing mutant Mrp constructs. Details of the methods for preparation and assays of membrane vesicles are described under Experimental Procedures. 2 mM Tris-succinate was used to energize the everted membrane vesicles. The arrow indicates the time at which the addition of 10 mM NaCl was made. The assay buffer contained BTP-chloride buffer, which contains 140 mM choline chloride, 10mM Bis Tris propane and 5 mM magnesium chloride and was adjusted to pH 8.0, 8.5 or 9.0 with sulfuric acid.

Figure S4. Immunoblot analysis of everted membrane vesicles from *E. coli* KNabc transformants. For each sample, thirty micrograms of protein was loaded on to the SDS-PAGE gels. Mrp subunits were detected with antibodies against unique epitope tags linked to their carboxy-terminus or, in the case of MrpE and MrpF, the subunits were detected with polyclonal antibodies raised against the proteins [main text (22)].

Figure S5. Detection of Mrp complexes by immunoblot analysis of BN-PAGE. Samples containing thirty micrograms of protein extracted from membranes using 1% DDM were loaded on BN-PAGE. BN-PAGE was carried according to instructions provided by the manufacturer (Invitrogen). Mrp subunits were detected with the same antibodies as used in the SDS-PAGE analysis. The results of the detection of MrpA and MrpD in MrpE T113A and P114G mutants in Figure S5H have been reported in a previous paper [main text (22)]. In a few instances, not every subunit was well detected, in the immunoblots in Fig. S5, in positions corresponding to monomeric or dimeric MrpA-to-MrpG complexes., Since those complexes are only formed when all seven Mrp proteins are present [main text (22)], the clear presence of multiple Mrp proteins at hetero-oligomer positions is scored as a “+” in the MrpA-G dimer or monomer column even if one or more other Mrp proteins are not well resolved.

Figure S6. Analysis of two mutants in category “complex formation” by two dimensional Blue native/SDS-PAGE. Thirty microgram of extracted proteins was loaded on one dimensional BN-PAGE. Then each lane was denatured in buffer containing of 50mM Tris-HCl (pH6.8), 2% SDS and 1% 2-mercaptoethanol for 30 min. The denatured BN-lanes were put on 2D SDS-PAGE gel and run. After transferred to nitrocellulose membrane, MrpE proteins were detected. Detail methods of BN-PAGE, SDS-PAGE and immunoblotting were described in Experimental Procedures. Values on top of the figure indicate the molecular weight of protein maker in 1D BN-PAGE.

TABLE S1. Bacterial strains and plasmids used in this study

Strain and plasmid	Genotype and description	Source and reference
E. coli Strains		
DH5αMCR	F$^-$ *mcrA*Δ*1* (*mrr-hsd RMS-mcrBC*) Φ80*dlacZ* Δ(*lacZYAargF*) *U169 deoR recA1 endA1 supE44 λthi-1 gyr-496 relA1*	Stratagene
KNabc	TG1 (Δ*nhaA* Δ*nhaB* Δ*chaA*)	(1)
Plasmids		
pGEM-5zf(+)	Cloning vector; Ap^R	Promega
pGEM-7zf(+)	Cloning vector; Ap^R	Promega
pGEMOF4mrp16	pGEM7Zf(+)+full *mrp* operon from *B. pseudofirmus* OF4	(2)
pGEMmrpTFCHS7	pGEMOF4mrp16, MrpA-T7 tag, MrpB-FLAG tag, MrpC-*c-myc* tag, MrpD-His$_7$ tag, MrpG-S-tag with mutation free	(2)
pTFCHS7MrpA-Y136A	pGEMmrpTFCHS7, MrpA-Y136A	This study
pTFCHS7MrpA- E140A	pGEMmrpTFCHS7, MrpA-E140A	This study
pTFCHS7MrpA-K223A	pGEMmrpTFCHS7, MrpA-K223A	This study
pTFCHS7MrpA-H230A	pGEMmrpTFCHS7, MrpA-H230A	This study
pTFCHS7MrpA-H230K	pGEMmrpTFCHS7, MrpA-H230K	This study
pTFCHS7MrpA-W232A	pGEMmrpTFCHS7, MrpA-W232A	This study
pTFCHS7MrpA-Y258A	pGEMmrpTFCHS7, MrpA-Y258A	This study
pTFCHS7MrpA -K299A	pGEMmrpTFCHS7, MrpA-K299A	This study
pTFCHS7MrpA-H345A	pGEMmrpTFCHS7, MrpA-H345A	This study
pTFCHS7MrpA-G392A	pGEMmrpTFCHS7, MrpA-G392A	This study
pTFCHS7MrpA-G392R	pGEMmrpTFCHS7, MrpA-G392R	This study
pTFCHS7MrpA -F405A	pGEMmrpTFCHS7, MrpA-F405A	This study
pTFCHS7MrpA-H700A	pGEMmrpTFCHS7, MrpA-H700A	This study
pTFCHS7MrpA-H700K	pGEMmrpTFCHS7, MrpA-H700K	This study
pTFCHS7MrpA-H700W	pGEMmrpTFCHS7, MrpA-H700W	This study
pTFCHS7MrpB-H34A	pGEMmrpTFCHS7, MrpB-H34A	This study
pTFCHS7MrpB-P37G	pGEMmrpTFCHS7, MrpB-G37G	This study
pTFCHS7MrpB-F40A	pGEMmrpTFCHS7, MrpB-F40A	This study
pTFCHS7MrpC-Q70A	pGEMmrpTFCHS7, MrpC-Q70A	This study
pTFCHS7MrpC-T75A	pGEMmrpTFCHS7, MrpC-T75A	This study
pTFCHS7MrpC-G82P	pGEMmrpTFCHS7, MrpC-G82P	This study
pTFCHS7MrpC-G82I	pGEMmrpTFCHS7, MrpC-G82I	This study
pTFCHS7MrpD-F135A	pGEMmrpTFCHS7, MrpD-F135A	This study

pTFCHS7MrpD-F136A	pGEMmrpTFCHS7, MrpD-F136A	This study
pTFCHS7MrpD-F136G	pGEMmrpTFCHS7, MrpD-F136G	This study
pTFCHS7MrpD-F136T	pGEMmrpTFCHS7, MrpD-F136T	This study
pTFCHS7MrpD-F136E	pGEMmrpTFCHS7, MrpD-F136E	This study
pTFCHS7MrpD-E137A	pGEMmrpTFCHS7, MrpD-E137A	This study
pTFCHS7MrpD-E137Q	pGEMmrpTFCHS7, MrpD-E137Q	This study
pTFCHS7MrpD-E137D	pGEMmrpTFCHS7, MrpD-E137D	This study
pTFCHS7MrpD-K219A	pGEMmrpTFCHS7, MrpD-K219A	This study
pTFCHS7MrpD-W228A	pGEMmrpTFCHS7, MrpD-W228A	This study
pTFCHS7MrpE-T113A	pGEMmrpTFCHS7, MrpE-T113A	This study
pTFCHS7MrpE-T113Y	pGEMmrpTFCHS7, MrpE-T113Y	This study
pTFCHS7MrpE-P114G	pGEMmrpTFCHS7, MrpE-P114G	This study
pTFCHS7MrpE-P114A	pGEMmrpTFCHS7, MrpE-P114A	This study
pTFCHS7MrpF-P28G	pGEMmrpTFCHS7, MrpF-P28G	This study
pTFCHS7MrpF-D32A	pGEMmrpTFCHS7, MrpF-D32A	This study
pTFCHS7MrpF-R33A	pGEMmrpTFCHS7, MrpF-R33A	This study
pTFCHS7MrpG-P81G	pGEMmrpTFCHS7, MrpG-P81G	This study
pTFCHS7MrpG-P81A	pGEMmrpTFCHS7, MrpG-P81A	This study

1. Nozaki, K., T. Kuroda, T. Mizushima, and T. Tsuchiya. (1998) *Biochim. Biophys. Acta* **1369,** 213-220.
2. Morino, M., Natsui, S., Swartz, T. H., Krulwich, T. A., and Ito, M. (2008) *J. Bacteriol.* **190**, 4162-4172

TABLE S2. Primers used in this study

Primer	Sequence (5'-3')	Accession number and corresponding sequence (nt)
MrpA-Y136A-F	CAGATAACTTAATTGTTCTTgcTGTATTTTGGG	EF468713 (1138-1157)
MrpA-Y136-R	AAGAACAATTAAGTTATCTGATAAGAC	EF468713 (1131-1157 (minus strand))
MrpA-E140A-F	GTTCTTTATGTATTTTGGGcATTAACTAGTTTAGC	EF468713 (1152-1186)
MrpA-E140-R	CCAAAATACATAAAGAACAATTAAGTTATCTG	EF468713 (1138-1169 (minus strand))
MrpA-K223A-F	CTGCTTGGTGCATTCACTgcATCAGCACAGTTC	EF468713 (1401-1433)
MrpA-K223-R	AGTGAATGCACCAAGCAGCACTA	EF468713 (1396-1418 (minus strand))
MrpA-H230A-F	CACTAAATCAGCACAGTTCCCATTCgcCATCTGGCTG	EF468713(1415-1451)
MrpA-H230K-F	CACTAAATCAGCACAGTTCCCATTCaAaATCTGGCTG	EF468713(1415-1451)
MrpA-H230-R	GAATGGGAACTGTGCTGATTTAGTGAATGCAC	EF468713(1408-1439(minus strand))
MrpA-W232A-F	CAGTTCCCATTCCACATCgcGCTGCCAGATGC	EF468713 (1428-1459)
MrpA-W232-R	GATGTGGAATGGGAACTGTGCTG	EF468713 (1423-1445 (minus strand))
MrpA-Y258A-F	CGATGGTTAAAGCAGGAATCgcTCTTGTTGCACG	EF468713 (1504-1537)
MrpA-Y258-R	GATTCCTGCTTTAACCATCGTTGCAG	EF468713 (1498-1523 (minus strand))
MrpA-K299A-F	CTGTCAGACAAAAAGACTTAgcAGGAATCCTTGCC	EF468713 (1627-1661)
MrpA-K299-R	TAAGTCTTTTTGTCTGACAGCAGATGTTG	EF468713 (1618-1646(minus strand))
MrpA-H345A-F	GGCTGCGATTTTCgcCTTAATAAATCATGCG	EF468713 (1772-1802)
MrpA-H345-R	GAAAATCGCAGCCATTATGGCAAAC	EF468713 (1760-1784 (minus strand))
MrpA-G392A-F	TTACGGTTTCATTAATTGcACTCGCCTCA	EF468713 (1909-1937)
MrpA-G392R-F	TTACGGTTTCATTAATTcGACTCGCCTCA	EF468713 (1909-1937)
MrpA-G392-R	AATTAATGAAACCGTAAATGTGACCGGC	EF468713 (1989-1925 (minus strand))
MrpA-F405A-F	GCCTTCCACCATTTAACGGGgcCTTAAGTAAGG	EF468713 (1945-1977)
MrpA-F405-R	CCCGTTAAATGGTGGAAGGCCTGCCATT	EF468713 (1937-1964 (minus strand))
MrpA-H700A-F	GCTTTTAATGCTTGCATTCTACgcCTTACCAGAACTTCG	EF468713 (2828-2866)
MrpA-H700K-F	GCTTTTAATGCTTGCATTCTACaAaTTACCAGAACTTCG	EF468713(2828-2866)
MrpA-H700W-F	GCTTTTAATGCTTGCATTCTACtggTTACCAGAACTTCG	EF468713(2828-2866)
MrpA-H700-R	GTAGAATGCAAGCATTAAAAGCAGTACTG	EF468713 (2821-2849(minus strand))
MrpB-H34A-F	TCTACCTATTCTTCGCCGGGgcTAATAATCCCGG	EF468713 (3296-3329)
MrpB-H34-R	CCCGGCGAAGAATAGGTAGACAGAAA	EF468713 (3290-3315 (minus strand))
MrpB-P37G-F	CTTCGCCGGGCATAATAATggCGGAGGAGGA	EF468713 (3306-3336)
MrpB-P37-R	ATTATTATGCCCGGCGAAGAATAGG	EF468713 (3300-3324 (minus strand))
MrpB-F40A-F	ATAATAATCCCGGAGGAGGAgcTATTGGGGGGTT	EF468713 (3317-3350)
MrpB-F40-R	TCCTCCTCCGGGATTATTATGCC	EF468713 (3314-3336 (minus strand))
MrpC-Q70A-F	CTTATTCTGACCCGTTGCCAgcAGCATTGATTTTG	EF468713 (3886-3920)
MrpC-Q70-R	TGGCAACGGGTCAGAATAAGTTGTTGC	EF468713 (3879-3905(minus strand))
MrpC-T75A-F	GCCACAAGCATTGATTTTGgCAGCAATTGT	EF468713 (3902-3931)
MrpC-T75-R	CAAAATCAATGCTTGTGGCAACGGGTCAG	EF468713 (3892-3920(minus strand))
MrpC-G82P-F	CAATTGTTATTAGTTTTccGGTCACTTCATTC	EF468713 (3925-3956)
MrpC-G82I-F	CAATTGTTATTAGTTTTattGTCACTTCATTC	EF468713 (3925-3956)
MrpC-G82-R	AAAACTAATAACAATTGCTGTCAAAATC	EF468713 (3914-3941(minus strand))
MrpD-F135A-F	CTTATTTAATCTATTCGTAgcCTTCGAGGTTATGC	EF468713 (4467-4501)
MrpD-F135-R	TACGAATAGATTAAATAAGTCTCCTG	EF468713 (4460-4485 (minus strand))
MrpD-F136A-F	TTTAATCTATTCGTATTCgcCGAGGTTATGCTG	EF468713 (4471-4503)
MrpD-F136G-F	TTTAATCTATTCGTATTCggCGAGGTTATGCTG	EF468713 (4471-4503)
MrpD-F136E-F	TTTAATCTATTCGTATTCgaaGAGGTTATGCTG	EF468713 (4471-4503)
MrpD-F136T-F	TCTATTCGTATTCacCGAGGTTATGCTGA	EF468713 (4476-4504)
MrpD-F136-R	GAATACGAATAGATTAAATAAGTCTCCTG	EF468713 (4460-4488(minus strand))
MrpD-E137A-F	CTTATTTAATCTATTCGTATTCTTCGcGGTTATGCTG	EF468713 (4467-4503)

MrpD-E137Q-F	CTTATTTAATCTATTCGTATTCTTCcAGGTTATGCTG	EF468713(4467-4503)
MrpD-E137D-F	CTTATTTAATCTATTCGTATTCTTCGAcGTTATGCTG	EF468713(4467-4503)
MrpD-E137-R	GAAGAATACGAATAGATTAAATAAGTCTCCTG	EF468713 (4460-4491(minus strand))
MrpD-K219A-F	TATTTTCTTAGTTGTATTTGCAATGgcGGGCGGTCTC	EF468713(4713-4749)
MrpD-K219-R	CATTGCAAATACAACTAAGAAAATAACCGCAA	EF468713(4706-4737(minus strand))
MrpD-W228A-F	GTCTCTTCCCCCTTTACTTCgcGCTTCCACGTT	EF468713 (4745-4777)
MrpD-W228-R	GAAGTAAAGGGGGAAGAGACCGCCCTT	EF468713 (4738-4764 (minus strand))
MrpE-T113A-F	GCATCTCTTATCTCTCTTgCTCCAGGTAC	EF468713 (5922-5950)
MrpE-T113Y-F	GCATCTCTTATCTCTCTTtaTCCAGGTAC	EF468713 (5922-5950)
MrpE-T113-R	AAGAGAGATAAGAGATGCTAGAAGAC	EF468713 (5914-5939 (minus strand))
MrpE-P114G-F	GCATCTCTTATCTCTCTTACTggAGGTACACTA	EF468713 (5922-5954)
MrpE-P114A-F	GCATCTCTTATCTCTCTTACTgCAGGTACACTA	EF468713 (5922-5954)
MrpE-P114-R	AGTAAGAGAGATAAGAGATGCTAGAAGAC	EF468713 (5914-5942 (minus strand))
MrpF-P28G-F	ATCCGTACATTAATCGGCggTACGATGTCTG	EF468713 (6145-6175)
MrpF-P28-R	GCCGATTAATGTACGGATAAAGCAAACG	EF468713 (6135-6162(minus strand))
MrpF-D32A-F	ATCGGCCCTACGATGTCTGcCCGCATCGTAGCGC	EF468713 (6157-6190)
MrpF-D32-R	AGACATCGTAGGGCCGATTAATGTACGG	EF468713 (6147-6174(minus strand))
MrpF-R33A-F	ATCGGCCCTACGATGTCTGACgcCATCGTAGCGC	EF468713 (6157-6190)
MrpF-R33-R	GTCAGACATCGTAGGGCCGATTAATGTACGG	EF468713 (6147-6177(minus strand))
MrpG-P81G-F	TTCGTTTTCCTAACTGCTggTGTAGCCGGAAT	EF468713 (6566-6597)
MrpG-P81A-F	TTCGTTTTCCTAACTGCTgCTGTAGCCGGAAT	EF468713(6566-6597)
MrpG-P81-R	AGCAGTTAGGAAAACGAATAAAATCG	EF468713 (6558-6583(minus strand))

Substituted nucleotides that were added to introduce point mutation sites are shown by a small letter.

Supplementary Figure S1.

A.

```
                                             o      TMS-1          i
BP_MrpA         ----------------------------MTVLHWATISPFLLAILIPFLYK------YAR 26
BH_MrpA         ----------------------------MTLLHWATVSPFLLAIFIPILYK------YTR 26
BS_MrpA         ----------------------------MQLLHLAILSPFLFAFIIPFLAK------YAK 26
SA_MnhA         ----------------------------MSLLHIAVILPLIFALIIPILYR------FFK 26
SM_PhaA1        MTRPASVLAGPKSRPPIHSQGDKTIEYAEKLLSVFILVPFAGS-LIAIFFP------SDQ 53
PA_MrpA         -----------------------------MILALIIALPFMGA-LLPLLAE------RGG 24
VC_MrpA         --------------------------------------------MKTGLDK------HTS 10
EC_NuoM         -----------------------------MLLPWLILIPFIGGFLCWQTER----FGVKV 27
RC_NuoM         ---------------------------MQNLLSIITFLPLAAAAVLAVVSRGSGPAADRN 33
EC_NuoL         ----------------------------MNMLALTIILPLIGFVLLAFSRGR-----WSE 27
RC_NuoL         ------------------------------MTTIILLAPLLGALIGGFGWRL-----ITE 25
EC_NuoN         -------------------------------MTITPQNLIALLPLLIVGLTV-------- 21
RC_NuoN         -------------------------------MTKAEFSLVLPEVLLAIYAMG-------- 21

                i               TMS-2          o                         o
BP_MrpA         RIHTGWFVLVLPLVLFIYFIQ------YLSITSTGGVVEHTIPWVPS--LGINFTVFVDG 78
BH_MrpA         SIHTGWFVLVLPLVLFGYFLS------LLPITSNGGVIHETIPWVPS--LGINFTVYVDG 78
BS_MrpA         RVHTGWFVLILPVLLFIYFLP------MIRMTQSGETLRSVLEWIPS--LGINFTVYIDG 78
SA_MnhA         RIHLGWFVLSVPIVIFIYMLT------LIKTTMSGNTVMKTLNWMPH--FGMNFDLYLDG 78
SM_PhaA1        RGAISWFAGAIALVCFLVTAG------LYPYVASGGVLHYRIDWVPE--LGLNFTLRMDG 105
PA_MrpA         RTLCAASAGVAPLIGLILLLG------QRGTVFAGQTLTASYSWLPE--IGINLSLRLDG 76
VC_MrpA         LTPNTVSARLLPFAVLPILLFG-----WVFYWHQTGGNAWQVTWVPG--LDLNLSFRLDG 63
EC_NuoM         PRWIALITMGLTLALSLQLWLQGGYSLTQSAGIPQWQSEFDMPWIPR--FGISIHLAIDG 85
RC_NuoM         AKWVALTATVVTFLVSLLLLAG------FDPANPGMQFVEDRAWI----MGLHYKLGVDG 83
EC_NuoL         NVSAIVGVGSVGLAALVTAFIGV----DFFANGEQTYSQPLWTWMSVGDFNIGFNLVLDG 83
RC_NuoL         KGALVVTTGLLFLSCILSWVV-------FLTLPAETQHIHLLDWIRSGALDTSWGIRLDR 78
EC_NuoN         VVVMLSIAWRRNHFLNATLSV----------IGLNAALVSLWFVGQAGAMDVTPLMRVDG 71
RC_NuoN         VLLFGVWTGKDRVAKPILWAS----------AVTMLALALIIGLGTGNDTAFGGLFIADG 71
                                                                       .  *
                          TMS-3         i    i          TMS-4              o
BP_MrpA         LSLLFALLITGIGTLVILYSIFYLSKKTESLNN------FYVYLLMFMGAMLGVVLSDN- 131
BH_MrpA         LSLLFALLITGIGTLVALYSIYYLSKKTEKLNN------FYVYLLMFMGAMLGVVLSDN- 131
BS_MrpA         LGLLFALLITGIGSLVTLYSIFYLSKEKEQLGP------FYVYLLMFMGAMLGVVLVDN- 131
SA_MnhA         LGLLFSLLISGIGSLVVLYSIGYLSK-SEQLGN------FYCYLLLFMGAMLGVVLSDN- 130
SM_PhaA1        FAWLFSALITAIGVLVVLYARYYMAE-EDPVPR------FFALFLAFMGSMLGVVLSGN- 157
PA_MrpA         LGFLFALLILGIGLLVILYARYYLSE-QEPMGR------FFAFLLLFMGSMLGVVLAEN- 128
VC_MrpA         LSFLFASLITGIGALIQIYALAYMKEKAARFS-------FHLYLTLFMLAMLGVVVSDN- 115
EC_NuoM         LSLLMVVLTGLLGVLAVLCSWKEIEKYQGFFHLN---------LMWILGGVIGVFLAID- 135
RC_NuoM         ISILFVMLTTFLMPLTIASAWHVETRVK-EYMIA---------FLVLEALMIGVFVALD- 132
EC_NuoL         LSLTMLSVVTGVGFLIHMYASWYMRGEEGYS-------RFFAYTNLFIASMVVLVLADN- 135
RC_NuoL         LTAIMLIVVTTVSALVHLYSWGYMAHDENWTHHEAYKARFFAYLSFFTFAMLMLVTSDN- 137
EC_NuoN         FAMLYTGLVLLASLATCTFAYPWLEGYNDNKDE--------FYLLVLIAALGGILLANAN 123
RC_NuoN         FARFSKVVILVSAAAVLAMSSDYMGRRGLLRFEYP--------ILIVLAVVGMMMMVSAG 123
                :      :           :                          .   :  :.
                o    Na     TMS-5       i             i           TMS-6
BP_MrpA         -LIVLYVFWELTSLASSLLISYWFH----REKSTYGAQKSMLITVFGGFAMLGGFSLLYV 186
BH_MrpA         -LIVLYVFWELTSLASSLLISYWFH----REKSTYGAQKSMLITVFGGFAMLGGFSLLYV 186
BS_MrpA         -VMVLYMFWELTSLSSFLLIGYWYK----REKSRYGAAKSLLITVSGGLCMLGGFILLYL 186
SA_MnhA         -VIILYLFWELTSFSSFLLISFWRE----RQASIYGAQKSLIITVFGGLSLLGGIILLAI 185
SM_PhaA1        -LILLAVFWELTSIVSFLLIGYWHH----NAHARDAARMALTITGTGGLAMFVGLIIIGK 212
PA_MrpA         -LLLMMLFWELTSLSSFLLIGFWGS----RSDARKGARMALTVTGGGGLALLAGVLLLGH 183
VC_MrpA         -ILLLFIFWELTTITSYLLIGFNHD----KPVSRKNALQSLLVTGAGGLALLAGLILLGL 170
EC_NuoM         -MFLFFFFWEMMLVPMYFLIALWGHKASDGKTRITAATKFFIYTQASGLVMLIAILALVF 194
RC_NuoM         -LVLFYLFFEAGLIPMFLIIGIWG-----GKERIYAAFKFFLYTFLGSVLMLVAMVAMYM 186
EC_NuoL         -LLLMYLGWEGVGLCSYLLIGFYYT----DPKNGAAAMKAFVVTRVGDVFLAFALFILYN 190
RC_NuoL         -LVQMFFGWEGVGVASYLLIGFYYK----KPSANAAAIKAFVVNRVGDFGFALGIMGLFF 192
EC_NuoN         HLASLFLGIELISLPLFGLVGYAFR----QKRSLEASIKYTILSAAASSFLLFGMALVYA 179
RC_NuoN         DLMSLYIGLELQSLALYVVAALRRD----SAVSSEAGLKYFVLGSLSSGLLLYGASLVYG 179
                 :  : .  *   .    : .               .         ..  :  .   :
                   o         o                        TMS-7
BP_MrpA         MTGTFSIRGIIENVDLVTSSEL---------FLPAMIL---------VLLGAFTKSAQFP 228
BH_MrpA         ITGTFSIREIIEQADAVLASTL---------FLPAMLL---------VLLGAFTKSAQFP 228
BS_MrpA         ITDSFSIREMVHQVQLIAGHEL---------FIPAMIL---------ILLGAFTKSAQFP 228
SA_MnhA         PTQSFSIQYMIQHASEIQNSPF---------FIFAMIL---------IMIGAFTKSAQFP 227
SM_PhaA1        IVGSYELDAVLASGDAIRNHPL---------YGTVLVL---------VLLGALTKSAQFP 254
PA_MrpA         IAGSFELTEVLAAGELIRAHAL---------YPLVLIL---------ILLGVFTKSAQFP 225
VC_MrpA         MANSYQISVIIEHADHIAQHPW---------FMPSLIL---------VLLGAFTKSAQFP 212
EC_NuoM         VHYNATGVWTFNYEELLNTP-------MSSGVEYLLMLG--------FFIAFAVKMPVVP 239
RC_NuoM         MAGTTDIVTLMSFDFPHADLPFLGWWTLTGGVQTLLFLA--------FFASFAVKMPMWP 238
EC_NuoL         ELGTLNFREMVELAPAHFADGN---------NMLMWAT-------LMLLGGAVGKSAQLP 234
RC_NuoL         LTDSIDMDVIFASAPELAKTEL---------HFLAWEFNAANLLAVLLFIGAMGKSAQLF 243
EC_NuoN         QSGDLSFVALGKNLGDGMLNEP---------LLLAGFG--------LMIVGLGFKLSLVP 222
RC_NuoN         FAGTTTFSGIITVVEQGHLPIG---------LLFGLVF---------LLAGLAFKVSAVP 221
                                                               .: .   * .
                 Q i        i          TMS-8 Na         o  o        TMS-9
BP_MrpA         FHIWLPDAMEAPTPVSAYLHSATMVKAGIYLVARLTP--VFAGSAEWFWLLTGFGVVTLL 286
BH_MrpA         FHIWLPDAMEAPTPVSAYLHSATMVKAGLYLVARLTP--VFGGTPEWFWLLAGFGIITLC 286
BS_MrpA         FYIWLPDAMEAPTPVSAYLHSATMVKAGIYVIARFSP--IFAFSAQWFWIVSLVGLFTMV 286
SA_MnhA         FYIWLPDAMEAPTPVSAYLHSATMVKAGLYLIARMTP--IFAASQGWVWTVTLVGLITLF 285
SM_PhaA1        FHFWLPHAMAAPTPVSAYLHSATMVKAGVFLLVRFWP--VMAGTEAWFWIVGLAGLTTLL 312
PA_MrpA         FHFWLPHAMAAPTPVSAYLHSATMVKAGVFLLARLYP--ALAGTEWFFYLVSLSGLVTLL 283
VC_MrpA         FHFWLPNAMAAPTPVSAYLHSATMVKAGIYLLARLSP--IYASSDFWFYCLTIVGAVTAL 270
EC_NuoM         LHGWLPDAHSQAPTAGSVDLAGILLKTAAYGLLRFSLPLFPNASAEFAPIAMWLGVIGIF 299
RC_NuoM         VHTWLPDAHVQAPTAGSVVLAAVLLKMGGYGFLRFSLPMFPVGAETMTTFVFILSAVAIV 298
EC_NuoL         LQTWLADAMAGPTPVSALIHAATMVTAGVYLIARTHG--LFLMTPEVLHLVGIVGAVTLL 292
RC_NuoL         LHTWLPDAMEGPTPVSALIHAATMVTAGVFLVCRMSP--LFEYAPEAKMMVVYVGAVTAF 301
EC_NuoN         FHLWTPDVYQGAPAPVSTFLATASKIAIFGVVMRLFLYAPVGDSEAIRVVLAIIAFASII 282
RC_NuoN         FHMWTPDVYEGSPTPVTAFFATAPKLAAMALIARVVHDAFGQVPGEWGQILAALALASMY 281
                .  * ..:   .:.  :   :          . *         .          .
                       i    i        TMS-10           o      o    TMS-11   Q
BP_MrpA         WGSTSAVRQKDLKGILAFSTVSQLGLIMTLLGLGSAAIYFG-ESVDPAFYSFAIMAAIFH 345
BH_MrpA         WGSISAVRQKDLKSILAFSTISQLGLIMCLFGLGSATLHFDPTDSMIKFYATATLAAVFH 346
BS_MrpA         WGSFHAVKQTDLKSILAFSTVSQLGMIISMLGVSAAALHYG----HTEYYTVAAMAAIFH 342
SA_MnhA         WASLNATKQQDLKGILAFSTVSQLGMIMAMLGIGAISYHYQ--GDDSKIYAAAFTAAIFH 343
SM_PhaA1        LGAYFAIFQQDLKGLLAYSTISHLGLITVLLSLGSP---------------LAAVAAVFH 357
PA_MrpA         LGAGMALFQHDLKGLLAYSTISHLGLITLLFGLDSP---------------LSNVAAVFH 328
VC_MrpA         WCALLAFKQTDLKLMLAYSTNVALGKLTLLLGLGTE---------------VALTAAVLF 315
```

```
EC_NuoM         YGAWMAFAQTDIKRLIAYTSVSHMGFVLIAIYTGSQL---------------AYQGAVIQ 344
RC_NuoM         YTSLVALAQEDMKKLIAYSSVAHMGYVTMGIFAANQQ---------------GVDGAIFQ 343
EC_NuoL         LAGFAALVQTDIKRVLAYSTMSQIGYMFLALGVQAWD------------------AAIFH 334
RC_NuoL         FAATVGLVQNDIKRVIAYSTCSQLGYMFVAAGSGVYS------------------VAMFH 343
EC_NuoN         FGNLMALSQTNIKRLLGYSSISHLGYLLVALIALQTG-------------EMSMEAVGVY 329
RC_NuoN         LGAIAGIGQRDIKRLMAYSSISHMGFGLLGLAAGTAAG---------------VESMLLY 326
                     .  * ::* ::.:::   :*                                 .
                                    i          i        TMS-12  C125
BP_MrpA         LINHATFKGSLFMTAG-IIDHETG--TRDIRKLGGLMAIMPVTFTVSLIGLASMAG---- 398
BH_MrpA         LINHATFKGSLFMTVG-IIDHETG--TRDIRKLGGLMAIMPVTFTVSLIGLAAMAG---- 399
BS_MrpA         LINHATFKGSLFMAVG-IIDHETG--TRDIRKLGGLMAIMPITFTISLIGTFSMAG---- 395
SA_MnhA         LINHATFKGALFMITG-AVDHSTG--TRDVKKLGGLLTIMPISFTITVITALSMAG---- 396
SM_PhaA1        IVNHATFKASLFMAAG-IIDHESG--TRDIRRLGGLFHFMPITATLAMVASAAMAG---- 410
PA_MrpA         IINHATFKASLFMAAG-IIDHETG--SRDMRLINGMWKYLPHTAVLAIVASLAMAG---- 381
VC_MrpA         IFAHSFYKAALFMVVG-NIDKATG--TREREKLGNLKSVLLLSLIAAVIAALSKSG---- 368
EC_NuoM         MIAHGLSAAGLFILCG-QLYERIH--TRDMRMMGGLWSKMKWLPALSLFFAVATLG---- 397
RC_NuoM         MLSHGFISGALFLCVG-VIYDRMH--TREIAAYGGLVNRMPAYALIFMFFTMANVG---- 396
EC_NuoL         LMTHAFFKALLFLASGSVILACHH--EQNIFKMGGLRKSIPLVYLCFLVGGAALS----- 387
RC_NuoL         LLTHAFFKAMLFLGAGSVIHAMHH--EQDMRNYGGLRKKIPFTFAIMMIGTLAITGVGIP 401
EC_NuoN         LAGYLFSSLGAFGVVSLMSSPYRGPDADSLFSYRGLFWHRPILAAVMTVMMLSLAG---- 385
RC_NuoN         MTIYIVMNVGTFAFILSMERDGKP--VTEIAALNMLSKTDPVKAFALLVLLFSLAG---- 380
                :  :       *                .      :            .   :
                        o                            o        TMS-13
BP_MrpA         -------LPPFNGFLSKEMFFTALLRATEMNAFNMETFGIIIVVLAWIASVFTFLYCLIM 451
BH_MrpA         -------LPPFNGFLSKEMFFSGVVTATTLGIFNMETWGFLFPVLAWVASVFTFVYCAIM 452
BS_MrpA         -------LPPFNGFLSKEMFFTSMLRVTHFDLFNVQTWGVLFPLFAWIGSVFTFIYSMKL 448
SA_MnhA         -------VPPFNGFLSKESFLETTFTASQANLFSVDTLGYLFPIIGIVGSVFTFVYSIKF 449
SM_PhaA1        -------VPLLNGFLSKEMFFAEAIETHLVNPLD-----TVTPYVATIAGMFAVTYSLRF 458
PA_MrpA         -------VPLLNGFLSKEMFFGETLSQNLLGSFN-----WLVPAVATLAGVFSVAYSLRF 429
VC_MrpA         -------VAPMLGFLSKEYMYKSSVESGIA----------WISLVLLLINALMVALAIAL 411
EC_NuoM         -------MPGTGNFVGEFMILFGSFQVVPV-----------ITVISTFGLVFASVYSLAM 439
RC_NuoM         -------LPGTSGFVGEFLTLLGIFQVNTW-----------VALFATSGVILSAAYALWL 438
EC_NuoL         -----ALPLVTAGFFSKDEILAGAMANGHI----------NLMVAGLVGAFMTSLYTFRM 432
RC_NuoL         FFSIGGVPVGFAGYLSKDAIIESAFASGNG----------FAFYVLVAAAGMTSFYSWRL 451
EC_NuoN         -------IPMTLGFIGKFYVLAVGVQAHLW----------WLVGAVVVGSAIGLYYYLRV 428
RC_NuoN         -------VPPMLGFFAKFAVIKAAIGAGFV----------WVPVAAVVASVIGAFYYLRI 423
                            .:..:       .                          :       .
                  i                    i          TMS-14         o
BP_MrpA         FFKTFTGKFK--PENYDVKVHEAPIGMLISPVILGSLVIVFGFFPNILAYTIIEPAMQAV 509
BH_MrpA         FFRTFTGEFK--KENYDVHVHEAPMGMLISPVILGSLVVIFGLFPNLLTYTIIEPAVTAI 510
BS_MrpA         LFKTFRGNYQ--PEQLEKQAHEAPVGMLVPPVILVALAVSLFFFPNILSYSLIEPAMNSI 506
SA_MnhA         IMHIFFGQYK--PEQLPKKAHEVSILMLLSPAILATLVIVFGLFPGILTNSIIEPATSSI 507
SM_PhaA1        IHGVFFGRPP--ADLPRKP-HEPPRWMRAPLDFLVLACLVVGIIPAQTIGPFLHTAVLSV 515
PA_MrpA         IHDVFFNGEP--ARLPKFPPHEPPRYMKVPVEILVFLCLLVGMLPAYTVAPLLAAAASAS 487
VC_MrpA         LYKPFFGQATKESESHPPKAIEQKKSLWLPAMGLAIASFLLPVFALDWINQHLVIPAVMA 471
EC_NuoM         LHRAYFGKAKSQIASQELP--GMSLRELFMILLLVVLLVLLGFYP--------------- 482
RC_NuoM         YRRVVFG-ELVKESLKTIS--DMTTREKAIFAPLVAMTLLLGVYP--------------- 480
EC_NuoL         IFIVFHGKEQ-----IHAHAVKG-VTHSLPLIVLLILSTFVGALIVPPLQG--------- 477
RC_NuoL         IFLTFYGEARG-DHHKHDHAHESPAVMLAPLALLAVGSVLAGMVWYHSFFGDKVASFFNL 510
EC_NuoN         AVSLYLHAPE--QPGRDAPSNWQYSAGGIVVLISALLVLVLGVWP--------------- 471
RC_NuoN         VYFMYFGEKS-------APLDGRMPALQFAFLVLAAVAMLGGAIN--------------- 461

                                   o          TMS-15       i
BP_MrpA         L-PTVLADGELFHVNIYMWHGFNAELFMTMGVVAAGIILFLMMKNWAKAAFYMK---ERD 565
BH_MrpA         LGADVFGGGE---ANIYLWHGFNPELFMTIAVVAFGTLIFMNMKKWQQTAFYLR---ERD 564
BS_MrpA         Y-PTLLDGHEKFHVHISQWHGVTTELLMTAGIVVIGTIGYLSLNKWKGIYKLFP---SKL 562
SA_MnhA         N-HTVIDDVE-----FHMFHGLTPAFLSTLVIYILGILLIVTFSYWVKLLQRQP---GKL 558
SM_PhaA1        L-REGTPDYS-----LSVWHGWNIPLIMSFVALSGGIGLYFLMRSYLATAVEGPPVFRLL 569
PA_MrpA         L-GGQLPEYS-----LAIWHGFNLPLLMSVVALVGGVLVYVLRKPLFNWYAGLPEVDAKL 541
VC_MrpA         MDPNSVPQAA------KLWQGFNIPLALSGITLVLGGVLYLNYATLVTWLTRLV---KPL 522
EC_NuoM         ------------------------------------------------------------
RC_NuoM         ------------------------------------------------------------
EC_NuoL         -------------------------VLPQTTELAHGSMLT-------------------- 492
RC_NuoL         PAAAHGEAHG-----TEHATEGHVPEAAMTAEAAHEAAMAGTMAMAEPAAEHAVAKAPQG 565
EC_NuoN         ------------------------------------------------------------
RC_NuoN         ------------------------------------------------------------

                              i       TMS-16        o
BP_MrpA         PLNWFYDS-SLSGVITGSQFVTRIQMTGLLRDYFAYMIVFMILLLGYTMFRYDAFAIDTT 624
BH_MrpA         PLNRVYDS-SLDWLIKGSQGITRVQMTGMLRDYFAYMCVFMILLFGYTMVRYNAFAIDTV 623
BS_MrpA         TLNRLYDK-LLTMMEKGSYRVTKQYMTGFLRDYLLYIFAGFIILIGGAFAIKGGFSFKTE 621
SA_MnhA         TFNYWYNR-SANVIPNYSEKMTNSYVTDYSRNNLVIIFGALILLT-FVTIFSVPFNINFK 616
SM_PhaA1        QGQRIFERVLVTLSWKWARWLEQRLGTRRLQPQMR-LLVFLALAAGASPLLLGNFELPPL 628
PA_MrpA         VFEQQVQR-VVALAARLTAWLENGSLQRYLAWLLGAALVVVAVELAPLARLTGSRGLTP- 599
VC_MrpA         PKAEQMFDAVLAYLATLASWQTQMLQQKRSSGYMLLFFAVLALILIYQPLPLP--ATFSA 580
EC_NuoM         ------------------------------------------------------------
RC_NuoM         ------------------------------------------------------------
EC_NuoL         --------------------LEITSGVVAVVGILLAAWLWLGKRTLVTSIANSAPGRLLG 532
RC_NuoL         AIFMAETNHVIHDAHGVPDWVKLSPFGAMVTGFFFAWLYYIGDKPLPGRTARALPG--LY 623
EC_NuoN         ------------------------------------------------------------
RC_NuoN         ------------------------------------------------------------

                   o         TMS-17         i  i        TMS-18       o o
BP_MrpA         NVTEIA-PYIWVITIVFIVATLSIPFINKR-ITAVVVVGVIGFLLALLFVVFRAPDLALT 682
BH_MrpA         NVSVIS-PYMWVLTLVFIGSTLAIPFINHR-ITAIIVVGVIGFLLALFFVIFRAPDLALT 681
BS_MrpA         GMAKIG-VYEIILTLVMISATVATVFARSR-LTAIIALGVVGYTLALFFVIFRAPDLALT 679
SA_MnhA         DVSPIR-IFEVCIVILLLSAAFLILFAKSR-LFSIIMLSAVGYAVSVLFIFFKAPDLALT 674
SM_PhaA1        VIRGIDPAFALLWAIGIACAIGSAYQAKFHRLASLVLLGGAGLVTCITFVWLSAPDLAVT 688
PA_MrpA         -LDGITALGMLVLAF---SGLATALFHRMR-LVALLILSVAGLLVALAFARFSAPDLALT 654
VC_MrpA         SLFDVH-FYEVAIALALIASALLCVLSTSR-LLSVLALGMAGFMTTLVFMIYSAPDVAKT 638
EC_NuoM         ----------------------------------QPILDTSHSAIGNIQQWFVNSVTTTR 508
RC_NuoM         ----------------------------------SLVTDLIGPSVAHLVQNYHADLGTLA 506
EC_NuoL         TWWYNAWGFDWLYDKVFVKPFLGIAWLLKRD----PLNSMMN-IPAVLSRFAGKGLLLSE 587
RC_NuoL         RFLLNKWYFDELFDLLFVNPAKSLGRKLWKGGDGAVIDGAINGLALGWIPFFTRVAGRIQ 683
EC_NuoN         ----------------------------------QPLISIVRLAMPLM------------ 425
RC_NuoN         ------------------------------------MAGVEGAAQAAAASLVN------- 478
                                                      .
                    TMS-19       Q  i          i          TMS-20          o
BP_MrpA         QLLIETVTVLLLMLAFYHLPELRKEE-FKPRFNVLNLIISIGVGFFITAIALSSLALGNE 741
BH_MrpA         QLLVETVTVVLFMLAFYHLPELRKEK-FTPRFNLVNLFISIGVGGLVTLIALSSFALGTE 740
BS_MrpA         QLVIETISVALFLLCFYHLPKLRLKT-KTRTFRMTNFIISLGVGVIVTLLGIASSSQRTK 738
SA_MnhA         QFVVESISTALFLLCFYHLPNLNRYN-EKRSFQLTNALIAGGVGLSVIIIGLIAYG---N 730
```

```
SM_PhaA1        QLLVEIVTTVLILLGLRWLPKRIEEP-VAAKTSRSGSA---------------------- 725
PA_MrpA         QLSVEVVTMVLLMLALYFLPSRTPAE-SSSLRGLRDVALAGGVGMLVALLAYAVLT---- 709
VC_MrpA         LLLVETLMVVFVVLLMRHMPYLSTVARHSVARRTLNAVIASVIGASVTLILLNITAHP-- 696
EC_NuoM         P----------------------------------------------------------- 509
RC_NuoM         QATAGN------------------------------------------------------ 512
EC_NuoL         NGYLRWYVASMSIGAVVVLALLMVLR---------------------------------- 613
RC_NuoL         SGYLFHYAFAMVLGIVALMFWVVRTGGMN------------------------------- 712
EC_NuoN         ------------------------------------------------------------
RC_NuoN         ------------------------------------------------------------

                                                 o          TMS-21     i
BP_MrpA         AGIEPISQFFIENSKELAGGYNMVNVILVDFRGLDTMLEVLVLGIAALGVIALIKLRMTG 801
BH_MrpA         AGLGTISEYFIENSKELAGGYNMVNVILVDFRGLDTLLEVLVLGIAALGVIAMIKLRMTG 800
BS_MrpA         D---SIASFFVKHSHDLGGGDNVVNVILVDFRGFDTMFEITVLTIAALGIYSMIKTKVKE 795
SA_MnhA         RHFESISKFYQEHVYDLAHGKNMVNVILVDFRGMDTLFESSVLGIAGLAVYTMIKLRKKR 790
SM_PhaA1        ------------------------------------------------------------
PA_MrpA         RPYESIAGFFVENSVSGGGGYNVVNVILVDFRGFDTLGEISVLAIAGVGIYAMLAGFALH 769
VC_MrpA         -IDTTLSDYFAQQSVPGGHGRNIVNVILVDFRAFDTLGEVIVVIMASLVAISLLPKRTEQ 755
EC_NuoM         ------------------------------------------------------------
RC_NuoM         ------------------------------------------------------------
EC_NuoL         ------------------------------------------------------------
RC_NuoL         ------------------------------------------------------------
EC_NuoN         ------------------------------------------------------------
RC_NuoN         ------------------------------------------------------------

BP_MrpA         REDV-------------------------------------------------------- 805
BH_MrpA         REDV-------------------------------------------------------- 804
BS_MrpA         EGKSGE------------------------------------------------------ 801
SA_MnhA         QTQGNEVKNHE------------------------------------------------- 801
SM_PhaA1        ------------------------------------------------------------
PA_MrpA         KPTCDPQGRNWAHAKHPLILETLSRVLLPLALLISVFIFLRGHNLPGGGFIAGLVTAIAL 829
VC_MrpA         PQKIHSLIFATT-----------AHIVTALMLMFSLYLLLRGHNAPGGGFIGALIAVIGF 804
EC_NuoM         ------------------------------------------------------------
RC_NuoM         ------------------------------------------------------------
EC_NuoL         ------------------------------------------------------------
RC_NuoL         ------------------------------------------------------------
EC_NuoN         ------------------------------------------------------------
RC_NuoN         ------------------------------------------------------------

BP_MrpA         ------------------------------------------------------------
BH_MrpA         ------------------------------------------------------------
BS_MrpA         ------------------------------------------------------------
SA_MnhA         ------------------------------------------------------------
SM_PhaA1        ------------------------------------------------------------
PA_MrpA         VLQYIASGSQWVEQRLPLNYAAMAGAGVLIAGLTGLGSWLFGYPFLTSAFGHFELPLIGE 889
VC_MrpA         SLLLFAESPQYVRDRLHFSPLNIALFGILLSFMAGAMSVAVGLPFLTGLWWKEILPLG-- 862
EC_NuoM         ------------------------------------------------------------
RC_NuoM         ------------------------------------------------------------
EC_NuoL         ------------------------------------------------------------
RC_NuoL         ------------------------------------------------------------
EC_NuoN         ------------------------------------------------------------
RC_NuoN         ------------------------------------------------------------

BP_MrpA         --------------------------------------------
BH_MrpA         --------------------------------------------
BS_MrpA         --------------------------------------------
SA_MnhA         --------------------------------------------
SM_PhaA1        --------------------------------------------
PA_MrpA         IELATAMLFDLGVYLTVVGAALLILSYLGKLSLMPEPVAQEEAL 933
VC_MrpA         ----TPLLFDVGIYLAIIGGVMSMLLRVKEELD----------- 891
EC_NuoM         --------------------------------------------
RC_NuoM         --------------------------------------------
EC_NuoL         --------------------------------------------
RC_NuoL         --------------------------------------------
EC_NuoN         --------------------------------------------
RC_NuoN         --------------------------------------------
```

B.

```
BP_MrpB         -----------------------------------------------MKN---------- 3
BH_MrpB         -----------------------------------------------MKNNS-------- 5
BS_MrpB         -----------------------------------------------MNE---------- 3
SA_MnhB         -----------------------------------------------MKE---------- 3
VC_MrpA         FAQQSVPGGHGRNIVNVILVDFRAFDTLGEVIVVIMASLVAISLLPKRTEQP-------- 756
SM_PhaB         FLERAYREGGGTNVVNVILVDFRGFDTLGEIAVLCIVALTVFALLLRFRPQSDSLEAPEQ 79
PA_MrpA         FVENSVSGGGGYNVVNVILVDFRGFDTLGEISVLAIAGVGIYAMLAGFALHKPTCDPQGR 778

                                       i            TMS-1        o   o
BP_MrpB         -------------------LKSNDVLLHTLTRVVTFIILAFSVYLFFAGHNNPGGGFIGG 44
BH_MrpB         -------------------FKSNEVMLHTVTRIVTFIILSFSVYLFFAGHNDPGGGFIGG 46
BS_MrpB         -------------------QKTNDLILQTATKLVSFIILLFSFYLFLSGHNAPGGGFVGG 44
SA_MnhB         ----------------------NDVVLRTVTKLVVFILLTFGFYVFFAGHNNPGGGFIGG 41
VC_MrpA         -------------------QKIHSLIFATTAHIVTALMLMFSLYLLLRGHNAPGGGFIGA 797
SM_PhaB         QKVQNAFDDDHPDRAAGDSVAEYLFIPAVIMRWMFPVTGMLAAFLFLRGHDLPGGGFAAG 139
PA_MrpA         NWAH----------------AKHPLILETLSRVLLPLALLISVFIFLRGHNLPGGGFIAG 822
                                        .:  :  : :  :   :. :::: **: ***** ..
                 TMS-2         i            i        TMS-3           o
BP_MrpB         LMTASALLLMYLGFDMRSIKKAIPFDFTKMIAFGLLIAIFTGFGGLLVGDPYLTQYFEYY 104
BH_MrpB         LMTASAFLLMYLAFDMDSMKKALPFNFTALIAIGLLLAIFTGVSSMLAGDPFLTQYFRYF 106
BS_MrpB         LITSSSIVLLLLAYDLKTVRSLLPVNFIYVAGAGLLLAVLTGVGSFVFGAPFLTHTFGYF 104
SA_MnhB         LIFSSAFILMFLAFNVEEVLESLPIDFRILMIIGALVSSITAIIPMFFGKPFLSQYETTW 101
VC_MrpA         LIAVIGFSLLLFAESPQYVRDRLHFSPLNIALFGILLSFMAGAMSVAVGLPFLTGLWWKE 857
SM_PhaB         IAMSIGFILQYMSGGTRWVEERLRIHPLRWMSIGLLVATATGVGSWFFGYPFLTSHAQYA 199
PA_MrpA         LVTAIALVLQYIASGSQWVEQRLPLNYAAMAGAGVLIAGLTGLGSWLFGYPFLTSAFGHF 882
                :    .: *  :. .   : . : .        * *::  :.      * *:*:
                           o        TMS-4          i
BP_MrpB         QIPILGETELTTALPFDLGIYLVVIGIALTIILTIAEDDM------------------ 144
BH_MrpB         QLPILGETELTTALPFDLGIYLVVIGIALTIILTIAEDDA------------------ 146
BS_MrpB         QLPILGKTELATATIFDLGVYLVVVGITMTIIQTIGEEE------------------- 143
SA_MnhB         ILPILGQIHVSTITLFELGILFSVVGVIVTVMLSLSGGRS------------------ 141
VC_MrpA         ILPLG------TPLLFDVGIYLAIIGGVMSMLLRVKEELD------------------ 891
SM_PhaB         SLPVVGKFPLASAILFDLGVFSLVLGATVLILIALAHQSVRAPRAHAKAARSDKEAVR 257
```

```
PA_MrpA         ELPLIGEIELATAMLFDLGVYLTVVGAALLILSYLGKLSLMP------EPVAQEEAL- 933
                 :*:       :   *::*:   ::*  : ::  :

C.
                o      TMS-1          i   i         TMS-2         o
BP_MrpC         MEILMSITAGVLFMVGTYLILTKSLLRVVVGLILLSHGAHLLLLTMAGLQRGAPPLLHL- 59
BH_MrpC         MEILMSITVGVLFMVGTYMILTKSLLRVVLGLVLLSHGAHLLLLTMAGLQRGVPPLLSI- 59
BS_MrpC         MEILMAVLAGIIFMAATYLLLSKSLLRVIIGTALLSHGVHLMLLTMGGLKKGAAPILSE- 59
SA_MnhC         MEIIMIFVSGILTAISVYLVLSKSLIRIVMGTTLLTHAANLFLITMGGLKHGTVPIYEA- 59
VC_MrpC         MEGLWSIVVGVFVAAGIYLMLERHLLRLLFGLILLSSAVNLAIFTAGRLTPASPPLIEVG 60
PA_MrpC         MEALFAITLGLLTASGVYLLLRARTFPVVLGLTLISYAVNLFLFAMGRLGGSVAVIGTPG 60
SM_PhaC         MELILSAGIGTLTASGVYLLLRPRTYQVIIGLSLLSFAVNLFIFGMGRLRVNAPPILDPG 60
                ** :     * :   . *::*      ::.*  *:: ..:* ::  . *      :
                            o    TMS-3  C125        i
BP_MrpC         ---EATTYSDPLPQALILTAIVISFGVTSFLLVLAYRTYKEHKTDDLDQLRGSADE-- 112
BH_MrpC         ---DAPYFADPLPQALILTAIVISFGVTSFLLVLAYRTYKEHQTDDLDQLRGTADE-- 112
BS_MrpC         ---HAKSFVDPLPQALILTAIVISFGVTSCILVKAFRAYQELESDDMGQMRGNDQHE- 113
SA_MnhC         ---NVKSYVDPIPQALILTAIVIAFATTAFFLVLAFRTYKELGTDNVESMKGVPEDD- 113
VC_MrpC         ALLPAEGAANPLPQALILTAIVIGFGLLVFALMLFYRAYFETRSADVDEMRRSEEEE- 117
PA_MrpC         ------EYGDPLPQALVLTAIVIGFAMTAFVVVLALRGLGDLGSDHVDGRPDEEEPRP 112
SM_PhaC         GVGDLARYTDPVPQALVLTAIVIGFAMTALFLVVLLASRGFTGTDHVDGREQRGD--- 115
                         :*:****:******.*.     ::          : .:       :
D.
                             o       TMS-1         i         i     TMS-2
BP_MrpD         ------------MNNLVILPILIPFIVGSFLILFAKHH-----SLQRVISGFAVVGMLLV 43
BH_MrpD         ------------MSNLVILPILLPFIVGSFLILFAKHH-----GIQRFLTAITMVGLLLF 43
BS_MrpD         ------------MNNFVILAILIPILSAILLIFMTKNL-----MLMRIFSTAASAIGIVI 43
SA_MnhD         ----------MIESNMLVLTLVIPVITAILLVFIGKRP-----IIKRYVALGGTLLTLVA 45
VC_MrpD         -----------MTAIGLTLPVVISLLTAVAIFLAGRNR-----WLTNGLSVICSVINLFT 44
SM_PhaD         MWTEGSSAVTDWLDHLLILPILLPLAV-AAVLIPINERD---RTLKGAIGFASTLVVFIL 56
PA_MrpD         ------------MSHWLILPILLPLFAGSLLLLPLAER------WQRGLSLLAALALIPL 42
EC_NuoL         -------------MNMLALTIILPLIGFVLLAFSRG-------RWSENVSAIVGVGSVGL 40
RC_NuoL         ---------------MTTIILLAPLLGALIGGFGWR-------LITEKGALVVTTGLLFL 38
EC_NuoM         --------------MLLPWLILIPFIGGFLCWQTER----FGVKVPRWIALITMGLTLAL 42
RC_NuoM         ------------MQNLLSIITFLPLAAAAVLAVVSRGSGPAADRNAKWVALTATVVTFLV 48
EC_NuoN                 MTITPQNLIALLPLLIVGLTVVVVMLSIAWR---RNHFLNATLSVIGLNAAL 49
RC_NuoN         --------MTKAEFSLVLPEVLLAIYAMGVLLFGVWTGK---DRVAKPILWASAVTMLAL 49

                         o                         o         TMS-3
BP_MrpD         SIYLAVDVYQN------GITVLELGNWQAP----FGIVLVADLFATMMVILASIVGVVCL 93
BH_MrpD         SFYLATLVYQE------GIYTLQLGNWPAP----FGIVLVADMFATMMIILSSIVGVACL 93
BS_MrpD         SGILVQTVFTK------GIQTLSLGGWKAP----YGIVLAADQFASLLVLTTAIIGLLVG 93
SA_MnhD         AIINLANVVKH------GPIRVELGSWKAP----YSIVFVLDIFSALLIITSIIITAIVI 95
VC_MrpD         GALLTITIYTH------GPASIAFGQWPAP----FGIVFVADLLSVGMVMVTAIIGLVSV 94
SM_PhaD         SMILMRLAAAGT-GSLPGSGVYQLGNWPAP----FGIVLVLDRLSALMLCLTSGLALAAQ 111
PA_MrpD         SLLLIRTAASG------DLSVYALGNWAAP----FGIVLMLDRLAALMLLATAVLGSAAL 92
EC_NuoL         AALVTAFIGVDFFANGEQTYSQPLWTWMSVGDFNIGFNLVLDGLSLTMLSVVTGVGFLIH 100
RC_NuoL         SCILSWVV---FLTLPAETQHIHLLDWIRSGALDTSWGIRLDRLTAIMLIVVTTVSALVH 95
EC_NuoM         SLQLWLQGGYSLTQSAGIPQWQSEFDMPWIPRFGISIHLAIDGLSLLMVVLTGLLGVLAV 102
RC_NuoM         SLLLLAG------FDPANPGMQFVEDRAWI--MGLHYKLGVDGISILFVMLTTFLMPLTI 100
EC_NuoN         VSLWFVGQAGA---------------------MDVTPLMRVDGFAMLYTGLVLLASLATC 88
RC_NuoN         ALIIGLGTGNDT---------------------AFGGLFIADGFARFSKVVILVSAAAVL 88
                                                      :  * ::
                  i         i           TMS-4              o   Na(VFF) TMS-5
BP_MrpD         FFAFQTISSEREKYYFY-------PFYFFLLAGVNGAFLT--GDLFNLFVFFEVMLIASY 144
BH_MrpD         FFAFQTISSERERYYFY-------PFYLFLIAGVNGAFLT--GDLFNLFVFFEVMLIASY 144
BS_MrpD         LYSFRSVGEKRERSFYY-------SGVQFLLAGVSGAFLT--GDLFNMYVFFELLLIASY 144
SA_MnhD         LYSYQTIGIERERYYYY-------FSVLFMLIGIIGAFTT--GDIFNLFVFFEVFLMSSY 146
VC_MrpD         IYAIADLSAKLSYARYH-------ALLHVLLAGVYGAFLT--GDIFNLYVWFEVMLIASF 145
SM_PhaD         AYSMARW--HTAGHHFH-------SLFQLLVAGLNGAFLT--GDLFNLFVFFEMMLAASY 160
PA_MrpD         IYALRGE--DRLGKHFH-------ALFQFQLLGINGAFLT--GDLFNLFVFFEILLIASY 141
EC_NuoL         MYASWYMRGEEGYS-------RFFAYTNLFIASMVVLVLA--DNLLLMYLGWEGVGLCSY 151
RC_NuoL         LYSWGYMAHDENWTHHEAYKARFFAYLSFFTFAMLMLVTS--DNLVQMFFGWEGVGVASY 153
EC_NuoM         LCSWKEIEKYQGFFHLN---------LMWILGGVIGVFLA--IDMFLFFFFWEMMLVPMY 151
RC_NuoM         ASAWHVETRVK-EYMIA---------FLVLEALMIGVFVA--LDLVLFYLFFEAGLIPMF 148
EC_NuoN         TFAYPWLEGYNDNKDEF--------YLLVLIAALGGILLANANHLASLFLGIELISLPLF 140
RC_NuoN         AMSSDYMGRRGLLRFEYP--------ILIVLAVVGMMMMVSAGDLMSLYIGLELQSLALY 140
                  :                              :   .     .:  ::.  *      :
                             i       i        TMS-6             o
BP_MrpD         ILIVLGGT-----KYQLRESLKYVVINVFASILFIVGVAYIYSITGTLNMADLAVKVG-- 197
BH_MrpD         ALIVLGGT-----KYQLRESFKYVVINVFASILFIVGVAYLYSVTGTLNFADLAIRVA-- 197
BS_MrpD         MLIVLGGT-----KIQLRESLKYIVFNIVSSALFVIGVGFLYAVTGTLNMADLSVKIS-- 197
SA_MnhD         FLLVIGST-----KIQLQETIKYVLVNVVSSSFFVMGVAILYSVVGTLNLADISNKLANL 201
VC_MrpD         GLMVLDAN-----QQQIDGAIKYVLLNLISTLVFLLSIGLLYGATGTLNLADLHDKAQ-- 198
SM_PhaD         GLLLHGSG-----PLRVKAGLHYIAVNLAASALFLIGVSLIYGAAGTLNMADLATKLAAL 215
PA_MrpD         ALLLHGGG-----AERVRSGLHYVILNLVGSAFFLIAVGTLYGLTGTLNMADMAQKIAMA 196
EC_NuoL         LLIGFYYT----DPKNGAAAMKAFVVTRVGDVFLAFALFILYNELGTLNFREMVELAPAH 207
RC_NuoL         LLIGFYYK----KPSANAAAIKAFVVNRVGDFGFALGIMGLFFLTDSIDMDVIFASAPEL 209
EC_NuoM         FLIALWGHKASDGKTRITAATKFFIYTQASGLVMLIAILALVFVHYNATGVWTFNYEELL 211
RC_NuoM         LIIGIWG-----GKERIYAAFKFFLYTFLGSVLMLVAMVAMYMMAGTTDIVTLMSFDFPH 203
EC_NuoN         GLVGYAFR----QKRSLEASIKYTILSAAASSFLLFGMALVYAQSGDLSFVALGKNLGDG 196
RC_NuoN         VVAALRRD----SAVSSEAGLKYFVLGSLSSGLLLYGASLVYGFAGTTTFSGIITVVEQG 196
                 :                   :       .   :  .   :
                   o             TMS-7                 i  i          TMS-8
BP_MrpD         ELEQ--------TGVLNVIAVIFLVVFAMKGGLFPLYFWLPRSYFGPPAAIAALFGGLLT 249
BH_MrpD         ELEQ--------QGVLNVIAILFLVVFGMKGALFPLYFWLPRSYYGPPAAIAALFGGLLT 249
BS_MrpD         ESGQ--------TGLITVIGVLLLLVFGMKGGIFPLYFWLPGSYYAPPAAISALFGALLT 249
SA_MnhD         SAHD--------SGLVNIVFILFIFVFATKAGVFPMFVWLPSAYYAPPIPIIAFFGALLT 253
VC_MrpD         -SLA--------PEMKTMLAGLLLFAFSIKAALFPLFAWLPASYHTLPSAIVALFAALLT 249
SM_PhaD         EPRS--------RTLVEMGSAILGVAFLVKAGMWPLSFWLPTAYAAATPPVAGVF-AVLT 266
PA_MrpD         DAER--------APLLAAAGLLLLVVFALKAALLPLYFWLPRAYATASAPVAALF-AIMT 247
EC_NuoL         FADG-NNMLMWAT-------LMLLGGAVGKSAQLPLQTWLADAMAGPTPVSALIHAATMV 259
RC_NuoL         AKTE-LHFLAWEFNAANLLAVLLFIGAMGKSAQLFLHTWLPDAMEGPTPVSALIHAATMV 268
EC_NuoM         NTP-------MSSGVEYLLMLGFFIAFAVKMPVVPLHGWLPDAHSQAPTAGSVDLAGILL 264
RC_NuoM         ADLPFLGWWTLTGGVQTLLFLAFFASFAVKMPMWPVHTWLPDAHVQAPTAGSVVLAAVLL 263
EC_NuoN         MLNEP---------LLLAGFGLMIVGLGFKLSLVPFHLWTPDVYQGAPAPVSTFLATASK 247
RC_NuoN         HLPIG----------LLFGLVFLLAGLAFKVSAVPFHMWTPDVYEGSPTPVTAFFATAPK 246
                                      :      *     .  * .      .
                       o               o         TMS-9         i    i
BP_MrpD         KVGIYAIMRTFTLIFNHDP----GFTHTLILILAGLTMFFGVLGAVSQFDFKRILSYHII 305
BH_MrpD         KVGIYAIIRTFTLIFNHDP----SFTHTIILALAGFTMFFGVLGAVSQFDFKRILSYHII 305
BS_MrpD         KVGLYAITRVFTLIFIHDT----AFTHQLMIWLAALTVIFGVIGSLAYSNVMKIVIYNII 305
```

```
SA_MnhD         KVGVYAIARTLSLFFSDNV----SFSHYVILFLALLTIIFGCVGAVAYANIKKIILYNVM 309
VC_MrpD         KVGVYALLRVFTLVFPLDG----SGWQPVLLGIAALTMLTGVLGAASQYDIKKILSFHII 305
SM_PhaD         KVGIYVIIRLHLLVFGTAAGASSGFGQEWLVTGGMLTIAFGGIGVLASQAMGRLAGYSVL 326
PA_MrpD         KVGIYSILRVYTLVFGDAAGELANLAQAWLWPLALATLGLGAIGALAARTLQGLLAYLVV 307
EC_NuoL         TAGVYLIARTHG--LFLMT----PEVLHLVGIVGAVTLLLAGFAALVQTDIKRVLAYSTM 313
RC_NuoL         TAGVFLVCRMSP--LFEYA----PEAKMMVVYVGAVTAFFAATVGLVQNDIKRVIAYSTC 322
EC_NuoM         KTAAYGLLRFSLPLFPNAS----AEFAPIAMWLGVIGIFYGAWMAFAQTDIKRLIAYTSV 320
RC_NuoM         KMGGYGFLRFSLPMFPVGA----ETMTTFVFILSAVAIVYTSLVALAQEDMKKLIAYSSV 319
EC_NuoN         IAIFGVVMRLFLYAPVGDS----EAIRVVLAIIAFASIIFGNLMALSQTNIKRLLGYSSI 303
RC_NuoN         LAAMALIARVVHDAFGQVP----GEWGQILAALALASMYLGAIAGIGQRDIKRLMAYSSI 302
                      . *                        .                .  :  :
                   TMS-10         o        TMS-11           i
BP_MrpD         SQVGYMVMGLGIYTQ--LAIAGAIYYIAHHIIVKAALFLFAGATQRITG----------- 352
BH_MrpD         SQVGYMVMGLGIFTP--LALAGAIYYIAHHIIVKAALFLFAGATEKITG----------- 352
BS_MrpD         TAVGVILFGVAVHTP--ASIQGAIYYLIHDMLVKGALFMLAGTLIALTG----------- 352
SA_MnhD         IAVGVILVGVAMMTE--SGMIGAIYYTLHDMLVKLALFLLIGIMIKITG----------- 356
VC_MrpD         SQIGYMIMGLAIYTP--LAITGAIFYVIHHILVKGNLFLIGGLIGRKYG----------- 352
SM_PhaD         VSSGTLLAAVGLGHD--GMLAGALFYLVSSTLTIGAFFLLIELVERGRDAGADVLAVTME 384
PA_MrpD         VSAGTLLAGVALGSE--RALAASLYYLLHSTWIAGGLFLLADLVARQR------------ 353
EC_NuoL         SQIGYMFLALGVQ-----AWDAAIFHLMTHAFFKALLFLASGSVILACHH---------- 358
RC_NuoL         SQLGYMFVAAGSG-----VYSVAMFHLLTHAFFKAMLFLGAGSVIHAMHH---------- 367
EC_NuoM         SHMGFVLIAIYTGSQ--LAYQGAVIQMIAHGLSAAGLFILCG-QLYERIH---------- 367
RC_NuoM         AHMGYVTMGIFAANQ--QGVDGAIFQMLSHGFISGALFLCVG-VIYDRMH---------- 366
EC_NuoN         SHLGYLLVALIALQTGEMSMEAVGVYLAGYLFSSLGAFGVVSLMSSPYRG---------- 353
RC_NuoN         SHMGFGLLGLAAGTA--AGVESMLLYMTIYIVMNVGTFAFILSMERDGKP---------- 350
                   *    .                            *
                                     i          TMS-12                   o
BP_MrpD         --------TTDLKKMGGLLKTHPWLAWMFFISAISLAG-----------IPPLSGFFSKF 393
BH_MrpD         --------TTELKKMGGLLKTHPWLAWLFFISAISLAG-----------IPPLSGFFSKF 393
BS_MrpD         --------TASLHKMGGLIKRYPVLGWMFFISAISLAG-----------IPPLSGFVGKF 393
SA_MnhD         --------TADLRQFGGLIKRYPVLGWSFFIAALSLAG-----------IPPLSGFYGKF 397
VC_MrpD         --------TTQLTQLGGVYRAMPWLTFGFLLAAFSLAG-----------FPPLSGFWGKF 393
SM_PhaD         AYGDFDEDEEEEEVGAAIPGTMAVLGLCFCLCALLLAG-----------LPPLSGFIAKF 433
PA_MrpD         ------GDKAGDLVQGPALQNPRLLGGAFFIGAIAVAG-----------LPPLSGFFGKV 396
EC_NuoL         --------EQNIFKMGGLRKSIPLVYLCFLVGGAALS----------ALPLVTAGFFSKD 400
RC_NuoL         --------EQDMRNYGGLRKKIPFTFAIMMIGTLAITGVGIPFFSIGGVPVGFAGYLSKD 419
EC_NuoM         --------TRDMRMMGGLWSKMKWLPALSLFFAVATLG-----------MPGTGNFVGEF 408
RC_NuoM         --------TREIAAYGGLVNRMPAYALIFMFFTMANVG-----------LPGTSGFVGEF 407
EC_NuoN         ------PDADSLFSYRGLFWHRPILAAVMTVMMLSLAG-----------IPMTLGFIGKF 396
RC_NuoN         --------VTEIAALNMLSKTDPVKAFALLVLLFSLAG-----------VPPMLGFFAKF 391
                                              .                       .: .:
                                     o       TMS-13            i
BP_MrpD         ALILA-----------AFLNENYIIAAVALAVGLLTLFSMMKIFIYAFWGEQ---KHTEE 439
BH_MrpD         ALILA-----------GFEEGRYVIVTVALIVGLLTLFSMMKIFIYAFWGEQ---KHTEQ 439
BS_MrpD         KIAEG-----------GFAEGEFTISMLILLSSLLVLYSVLRIFIHAFWGEE---KETPK 439
SA_MnhD         FIVQS-----------TFERGFYLSGVIVLLSSLVVLYSVIRIFLQGFFGQP---KGYDL 443
VC_MrpD         LVIKA-----------SLAAELYWLAAIALLVGLLTIFSMTKIWNEVFWKDAPAALPQET 442
SM_PhaD         ALISGLFDMPAAELATAMSAADWTYVTLLILSGLAAMIAMNRIGIRTFWASI----EGTI 489
PA_MrpD         MLLQ----------SVAPGSQALALWSVVLGSGLVALVALSRAGSTLFWRTG----HTVL 442
EC_NuoL         EILAG-----------AMANGHINLMVAGLVGAFMTSLYTFRMIFIVFHGKEQ----IHA 445
RC_NuoL         AIIES-----------AFASGNGFAFYVLVAAAGMTSFYSWRLIFLTFYGEARGDHHKHD 468
EC_NuoM         MILFG-----------SFQVVP-VITVISTFGLVFASVYSLAMLHRAYFGKAKSQIASQE 456
RC_NuoM         LTLLG-----------IFQVNT-WVALFATSGVILSAAYALWLYRRVVFG-ELVKESLKT 454
EC_NuoN         YVLAVG-----------VQAHLWWLVGAVVVGSAIGLYYYLRVAVSLYLHAPE-QPGRDA 444
RC_NuoN         AVIKA-----------AIGAGFVWVPVAAVVASVIGAFYYLRIVYFMYFGEK------SA 434

                       i         TMS-14
BP_MrpD         QANFKVGKLLLPIVPLVALTIILGFAAEP------------------------------- 468
BH_MrpD         QAKMPIGKLLLPIAPLVVLTIVLGFGAEP------------------------------- 468
BS_MrpD         PNHRTAKGLLYPAAISLLLSLLFGLGTEW------------------------------- 468
SA_MnhD         NNKVDVKYLTTIAIVAVVITVLYGLSADY------------------------------- 472
VC_MrpD         LARSTLGLYALPILVLTLFSLVIGLAAQP------------------------------- 471
SM_PhaD         PRVVVIE--ITPVVVLLGACIFLSLQAGP------------------------------- 516
PA_MrpD         GSAELDHGRLFACILLLSAGPLLVFAAKP------------------------------- 471
EC_NuoL         HAVKG-VTHSLPLIVLLILSTFVGALIVPPLQG--------------------------- 477
RC_NuoL         HAHESPAVMLAPLALLAVGSVLAGMVWYHSFFGDKVASFFNLPAAAHGEAHGTEHATEGH 528
EC_NuoM         LPGMS-LRELFMILLLVVLLVLLGFYPQP------------------------------- 484
RC_NuoM         ISDMT-TREKAIFAPLVAMTLLLGVYPSL------------------------------- 482
EC_NuoN         PSNWQYSAGGIVVLISALLVLVLGVWPQP------------------------------- 473
RC_NuoN         PLDGRMPALQFAFLVLAAVAMLGGAINMAG------------------------------ 464
                                     .

BP_MrpD         -----------------------------------------------------------I 469
BH_MrpD         -----------------------------------------------------------I 469
BS_MrpD         -----------------------------------------------------------V 469
SA_MnhD         -----------------------------------------------------------L 473
VC_MrpD         -----------------------------------------------------------F 472
SM_PhaD         -----------------------------------------------------------A 517
PA_MrpD         -----------------------------------------------------------L 472
EC_NuoL         --VLPQTTELAHGSMLT----------------------------------------LEI 495
RC_NuoL         VPEAAMTAEAAHEAAMAGTMAMAEPAAEHAVAKAPQGAIFMAETNHVIHDAHGVPDWVKL 588
EC_NuoM         -----------------------------------------------------------I 485
RC_NuoM         -----------------------------------------------------------V 483
EC_NuoN         -----------------------------------------------------------L 474
RC_NuoN         -----------------------------------------------------------V 465

                 o
BP_MrpD         FQYSLQVADQILDPTIYIESVLKE------------------------------------ 493
BH_MrpD         FQYSLQVADELLNPSVYIESVLKE------------------------------------ 493
BS_MrpD         SPYVDQAAETLLNPEKYIEAVLKE------------------------------------ 493
SA_MnhD         YPMVKAGAETFYNPSTYVKAVLGGK----------------------------------- 498
VC_MrpD         YQFATLAAEQLLTPTAYIHAVLGGSK---------------------------------- 498
SM_PhaD         MRYMQATADDLLAPLTHSERVLSAPRAGSQ------------------------------ 547
PA_MrpD         LAYVQATAAQLHD-LDLYRQIITRGGAA-------------------------------- 499
EC_NuoL         TSGVVAVVGILLAAWLWLGKRTLVTSIANSAPGRLLGTWWYNAWGFDWLYDKVFVKPFLG 555
RC_NuoL         SPFGAMVTGFFFAWLYYIGDKPLPGRTARALPG--LYRFLLNKWYFDELFDLLFVNPAKS 646
EC_NuoM         LDTSHSAIGNIQQWFVNSVTTTRP------------------------------------ 509
RC_NuoM         TDLIGPSVAHLVQNYHADLGTLAQATAGN------------------------------- 512
EC_NuoN         ISIVRLAMPLM------------------------------------------------- 485
RC_NuoN         EGAAQAAAASLVN----------------------------------------------- 478

                          :
```

```
BP_MrpD         ------------------------------------------------------------
BH_MrpD         ------------------------------------------------------------
BS_MrpD         ------------------------------------------------------------
SA_MnhD         ------------------------------------------------------------
VC_MrpD         ------------------------------------------------------------
SM_PhaD         ------------------------------------------------------------
PA_MrpD         ------------------------------------------------------------
EC_NuoL         IAWLLKRD---PLNSMMN-IPAVLSRFAGKGLLLSENGYLRWYVASMSIGAVVVLALLM 610
RC_NuoL         LGRKLWKGGDGAVIDGAINGLALGWIPFFTRVAGRIQSGYLFHYAFAMVLGIVALMFWVV 706
EC_NuoM         ------------------------------------------------------------
RC_NuoM         ------------------------------------------------------------
EC_NuoN         ------------------------------------------------------------
RC_NuoN         ------------------------------------------------------------


BP_MrpD         ------
BH_MrpD         ------
BS_MrpD         ------
SA_MnhD         ------
VC_MrpD         ------
SM_PhaD         ------
PA_MrpD         ------
EC_NuoL         VLR--- 613
RC_NuoL         RTGGMN 712
EC_NuoM         ------
RC_NuoM         ------
EC_NuoN         ------
RC_NuoN         ------

E.
                                               o       TMS-1         i
BP_MrpE         ------------MAFQILLNLVIAVIWVNFQNSYTAVDFLIGYVVGIFILFVLRRFLRFD 48
BH_MrpE         ------------MALQILLNLVIAIIWMFLQNSFTFADFLVGYVVGIGILIALRRFMIFD 48
BS_MrpE         ------------MASQILLNVFLAFGWMFLSNSPSAAGFITGYILGMLSLFVFRRFFTRQ 48
SA_MnhE         ------------MAVQLVLNFIIAVFWLFVTNSYTTNNFVLGFIFGLVLVYLLHRVLPGR 48
VC_MrpE         -------------MIYLFLNLFLATAWMLLNGDYSSLQFLLGFVVGFWALRLSQPFGLKT 47
PA_MrpE         MISRSLPRPLLRVLPHPVLSVLLLLVWLLLVDSFAIGHWLLGAFLGVCIPLLTNRLLVGR 60
SM_PhaE         ---------MRTWFPYPLLSIALLLMWLLLSQSVTPGSIVLGLVVSTVLAWVTLNLQPAR 51
                                 .*.. :   *: .  . :    : * ...         .
                    i      TMS-2                o                      o
BP_MrpE         FYMRRIWAIIKLISLFFKELILANIDVIKIVLSPKMN-IQPGIVAVPTKLKTDWELSLLA 107
BH_MrpE         LYFRKVWAWIKLIVLFFKELVLANIDVTKIVLSPKLD-ITPGIVAVPTKLKSDWELTLLA 107
BS_MrpE         FHLWKLISIIKLCFIFIKELYLANVSVMKSVLSPKLN-IRPGIFGFKTELTKDWEITMLS 107
SA_MnhE         FYVITLYRIIKLVIIFLIELIKANFDVLKIIIKPSIK-NEPGFFVYHTDLKKDWQIVLLS 107
VC_MrpE         TYFRRFRAITTLILYFIYEMVVSVARVAWDVVTPKHL-SDPDIVYVPLDARSDLEITLLA 106
PA_MrpE         SQEWHPLLLLKLLVLVMWDILVANIQVARLTLGPIER-LRPAFVEVPIELENDLAISVLV 119
SM_PhaE         SRLHRWSRIAGFILRVVGDVIRSNIAVTLIILRAGRRPVNAGFMTVSLDLDDENALALLA 111
                           :   .. ::   :   *    : .      . :.    .   :  : :*
                TMS-3        i
BP_MrpE         SLISLTPGTLSMDFSDDNKYIYIHAIDVPNKEKMIRDIHDTFERAILEVTK---- 158
BH_MrpE         GLISLTPGTLSMDFSDDKKYIYIHSIHVPDKEAMIREIHDTFEKAILEVTA---- 158
BS_MrpE         LLITLTPGTLVMDISDDRTILYIHAMDIEDAEKAIFDIRESFEKAIQEVSR---- 158
SA_MnhE         NLITLTPGTVVLGVSDDRTKIYIHAIDFSTKEQEVESIKTSLEKIVREVGEI--- 159
VC_MrpE         NMVSLTPGTLSLDVSEDRQYLIVHAMFAPEHQAVINDIKNGLEKRILEVTRG--- 158
PA_MrpE         SIVSLTPGSVSSDLSDDRKTLLVHGLDVPDKAALIAEIKQRYEAPLKEIFPCSLT 174
SM_PhaE         CVVTATPGTAWLEYDRRQKILLFHVLDIEN-EDLWRKTITRYAADLKEIFE---- 161
                 ::: ***:     .  .  : .* :          .        : *:
F.
                                o          TMS-1         I    i       TMS-2
BP_MrpF         --------------MFQSILMIVLVVMSISLFVCFIRTLIGPTMSDRIVALDTFGINLIG 46
BH_MrpF         --------------MFETVLHVVLVIMSLSLAACFYRTIVGPTMSDRIVALDTFGINLIG 46
BS_MrpF         --------------MFTLILQIALGIMGVSTFLYVIRVIKGPTVPDRVVALDAIGINLIA 46
SA_MnhF         -------------MNHNVIIVIALIIVVISMLAMLIRVVLGPSLADRVVALDAIGLQLMA 47
VC_MrpF         MVLKNGYWRSLVDNSLPLAIHFAYLGLLLSLAMAFIRLVLGPSLADRVVALDLISFITIG 60
PA_MrpF         --------------MLAYVIPLCLALLGLALLLTLARLVKGPCLPDRALALDTLYVNAIA 46
SM_PhaF         -----------MELAVVWSVLVAQTMLALAMAFALYRMARGPRAQDRILGLDTLYINAML 49
                                   : .    : ::    . *   **   ** :.** : .  :
                        o     o        TMS-3    i
BP_MrpF         FIGVIMMLQETLAYSEVVLVISILAFIGSIALSKFIER--GVVFDRG----- 91
BH_MrpF         FIGIIMILQETIAYAEVILVLGILAFVGSIALAKFLER--GTIFDRSRDHR- 95
BS_MrpF         ITALVSILLKTSAFLDIILLLGILSFIGTIAFSKFLEK--GEIIENDRNR-- 94
SA_MnhF         VIALFSILLNIKYMIVVIMMIGILAFLGTAVFSKFMDK--GKVIEHDQNHTD 97
VC_MrpF         FIVVYSLDSGQQTLLDIALTLGLVAFLGTIAFARFIAKRKGEL--------- 103
PA_MrpF         LLVLYGIWRDSDLFFEAALLIAVLGFVGTVAVAKYMLR--GDIIE------- 89
SM_PhaF         MLITFGIRTANTVYFETALIIAVIGFASSIALAKFLMR--GEVIE------- 92
                .     :           : ::.:.* .: ..:::: :  * :

G.
                           i         TMS-1          o          o      TMS-2
BP_MrpG         -------MTAVEIIISIFVLIGGFLSLLGSIGIIRFPDVYGRLHAATKSATLGVISIMLA 53
BH_MrpG         -------MTAVEIIVSLFILLGAFLSFLASVGIIRFPDVYGRIHAATKSATLGVISIMVA 53
BS_MrpG         ------MIETAKVVVAVFILLGALICLIASFGVLRLPDVFTRAHAASKGSTLGVNMILLG 54
SA_MnhG         -----MIKIILISLALIFVIIGALISALAAIGLLRLEDVYSRAHAAGKASTLGAMSLLFG 55
VC_MrpG         ----------MSMLAALLLVLGTLFTLFASLGILRMPDLYTRMHAATKAGTAGLSLLLLA 50
PA_MrpG         -MTGEPLSLWLEIIVSAFLLLGSAFVLIGAIGLYRLPDFFMRLHGPTKATTLGVGGVIVA 59
SM_PhaG         MSHLTDLPPWAALLVCGLMLVGAATTLIGSLGLLRLPDFYARLHAPTIATSGGTILLCLA 60
                             :   ::::*     :.:.*: *: *.: * *..  . : *   : ..
                         i i          TMS-3     o
BP_MrpG         TFLFFFLVHGEFVGKLLTILFVFLTAPVAGMMMGRSAYRVGVPLWEKSTQDDLK--KMY 111
BH_MrpG         TFLYFLLIEGLFVGKILFTILFVFLTAPVAGLMISRSAYRIGVPLWDQSVQDDLK--KVY 111
BS_MrpG         VFFYLWFVTGELSAKILLGILFIFITSPIGGHLICRAAYNSGVKLDERSVQDDYNGIRNF 114
SA_MnhG         TFLYFIATQGFVNMQLIVAIIFVLITGPLSSHMIMKAAYNIKTPYTKKTKVDEIS----- 110
VC_MrpG         VALCMPEIG--VISRLVGIMLFIFLTAPVAAHLLGKVTQQAGYAFWRNQDAAKQK----- 103
PA_MrpG         SLIYFSNRQAGISLHELLISLFLFISAPVSAYMLAKAAVL-----QQLPLEKKTR----- 109
SM_PhaG         SILCFAVLQSRWVFHEVLIIFFVTVTTPVTLMLLGQATLYRDRFEEQQGVPRKQK----- 115
                  : :         : :   :*: :: *:   :: : :              .

BP_MrpG         EKKMKGSN-- 119
BH_MrpG         AKKRQT---- 117
BS_MrpG         VIKRKEDSYL 124
SA_MnhG         -EDLKDTKL- 118
VC_MrpG         -KAQK----- 107
```

```
PA_MrpG         GKPWEQ---- 115
SM_PhaG         PAPGEE---- 121
                    :
```

Figure S2

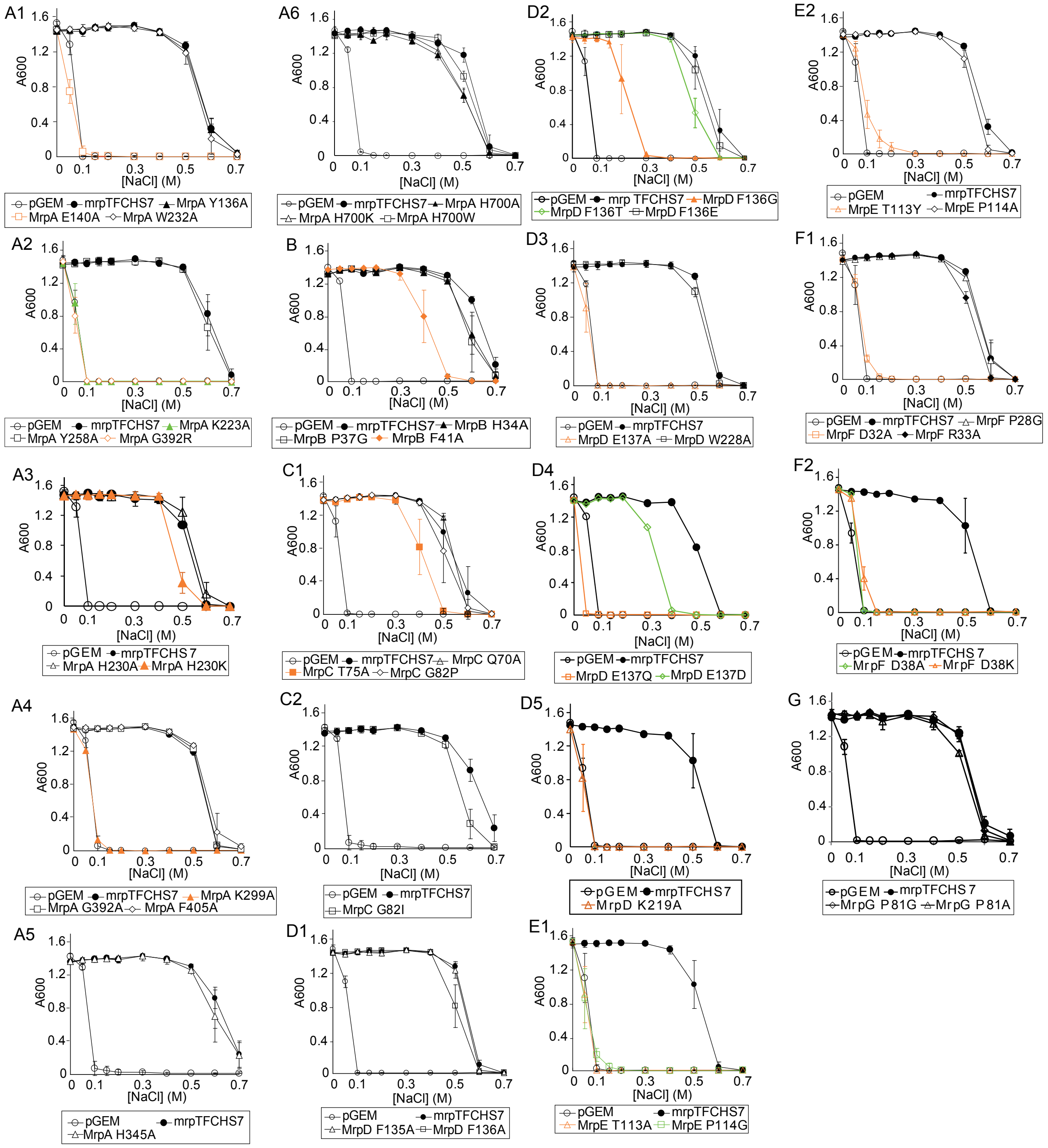

A1
A600
[NaCl] (M)
pGEM mrpTFCHS7 MrpA Y136A
MrpA E140A MrpA W232A
A2
pGEM mrpTFCHS7 MrpA K223A
MrpA Y258A MrpA G392R
A3
pGEM mrpTFCHS7
MrpA H230A MrpA H230K
A4
pGEM mrpTFCHS7 MrpA K299A
MrpA G392A MrpA F405A
A5
pGEM mrpTFCHS7
MrpA H345A
A6
pGEM mrpTFCHS7 MrpA H700A
MrpA H700K MrpA H700W
B
pGEM mrpTFCHS7 MrpB H34A
MrpB P37G MrpB F41A
C1
pGEM mrpTFCHS7 MrpC Q70A
MrpC T75A MrpC G82P
C2
pGEM mrpTFCHS7
MrpC G82I
D1
pGEM mrpTFCHS7
MrpD F135A MrpD F136A
D2
pGEM mrp TFCHS7 MrpD F136G
MrpD F136T MrpD F136E
D3
pGEM mrpTFCHS7
MrpD E137A MrpD W228A
D4
pGEM mrpTFCHS7
MrpD E137Q MrpD E137D
D5
pGEM mrpTFCHS7
MrpD K219A
E1
pGEM mrpTFCHS7
MrpE T113A MrpE P114G
E2
pGEM mrpTFCHS7
MrpE T113Y MrpE P114A
F1
pGEM mrpTFCHS7 MrpF P28G
MrpF D32A MrpF R33A
F2
pGEM mrpTFCHS7
MrpF D38A MrpF D38K
G
pGEM mrpTFCHS7
MrpG P81G MrpG P81A
0
0.4
0.8
1.2
1.6
0.1
0.3
0.5
0.7

Figure S3

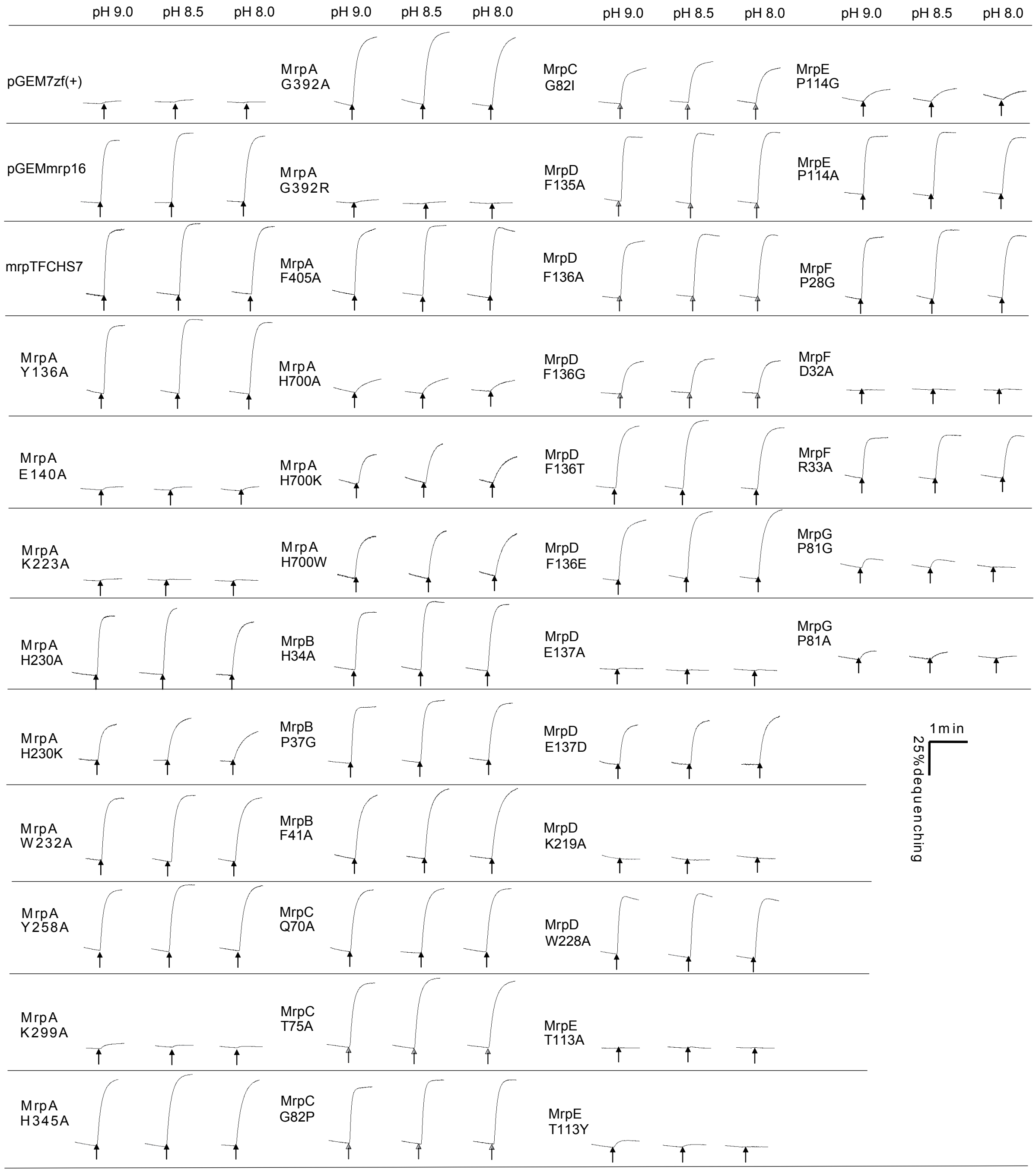

pH 9.0
pH 8.5
pH 8.0
pH 9.0
pH 8.5
pH 8.0
pH 9.0
pH 8.5
pH 8.0
pH 9.0
pH 8.5
pH 8.0
pGEM7zf(+)
MrpA G392A
MrpC G82I
MrpE P114G
pGEMmrp16
MrpA G392R
MrpD F135A
MrpE P114A
mrpTFCHS7
MrpA F405A
MrpD F136A
MrpF P28G
MrpA Y136A
MrpA H700A
MrpD F136G
MrpF D32A
MrpA E140A
MrpA H700K
MrpD F136T
MrpF R33A
MrpA K223A
MrpA H700W
MrpD F136E
MrpG P81G
MrpA H230A
MrpB H34A
MrpD E137A
MrpG P81A
MrpA H230K
MrpB P37G
MrpD E137D
1min
25%dequenching
MrpA W232A
MrpB F41A
MrpD K219A
MrpA Y258A
MrpC Q70A
MrpD W228A
MrpA K299A
MrpC T75A
MrpE T113A
MrpA H345A
MrpC G82P
MrpE T113Y

Figure S4

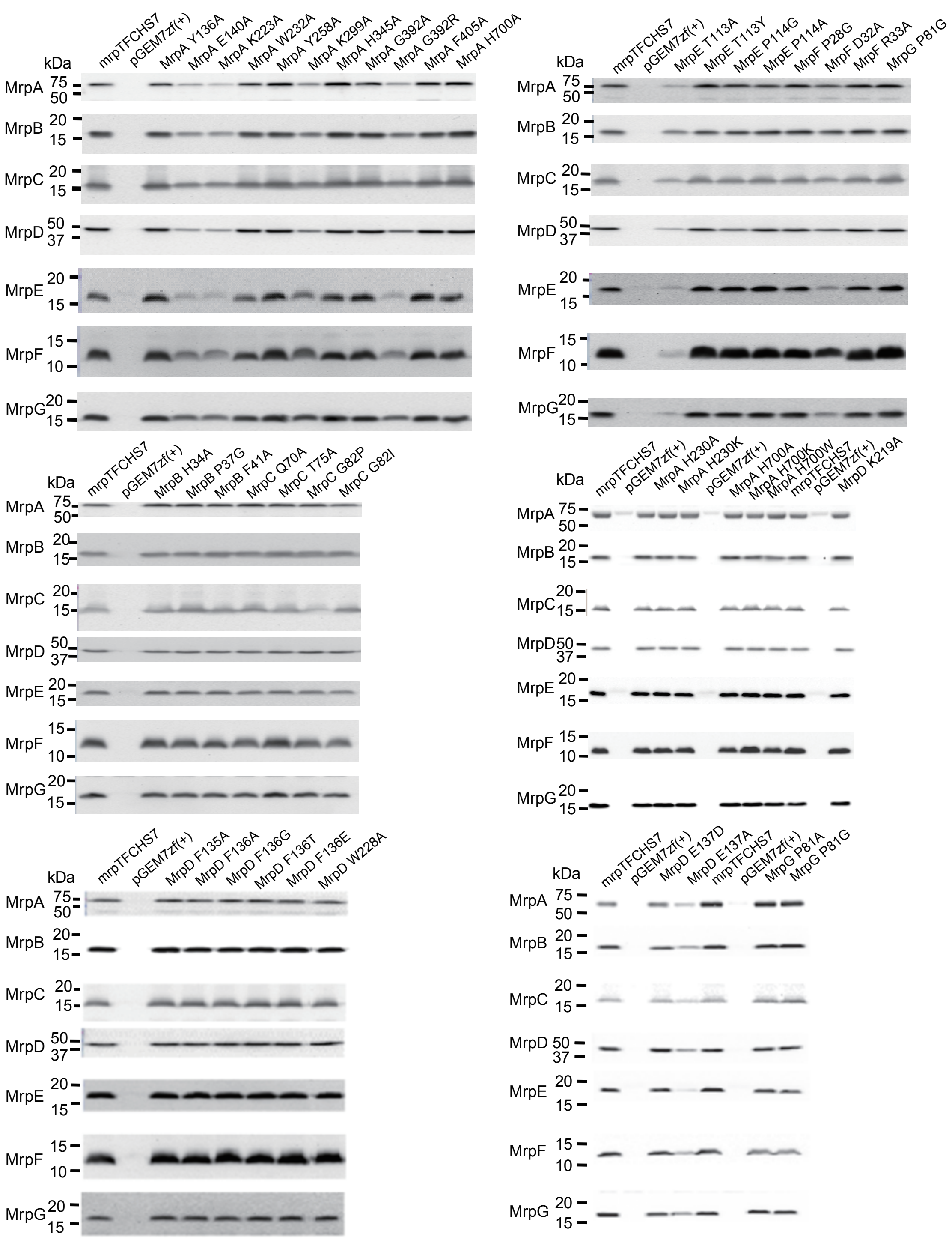
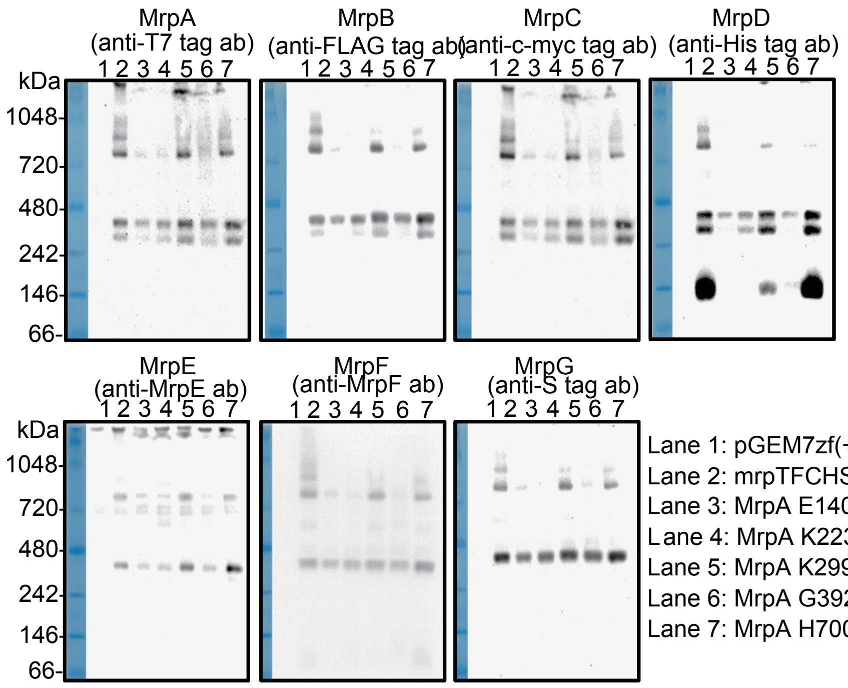
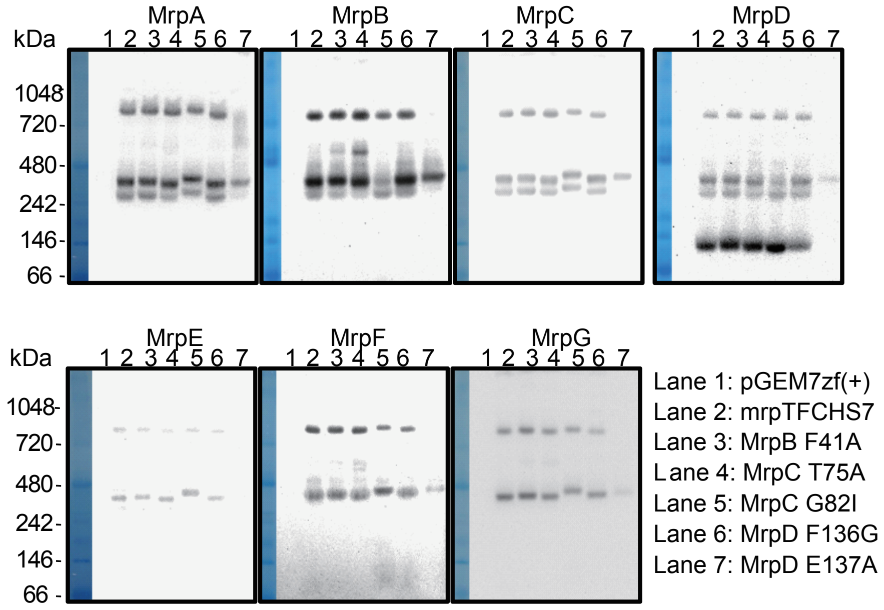

mrpTFCHS7
pGEM7zf(+)
MrpA Y136A
MrpA E140A
MrpA K223A
MrpA W232A
MrpA Y258A
MrpA K299A
MrpA H345A
MrpA G392A
MrpA G392R
MrpA F405A
MrpA H700A
kDa
MrpA 75 50
MrpB 20 15
MrpC 20 15
MrpD 50 37
MrpE 20 15
MrpF 15 10
MrpG 20 15
mrpTFCHS7
pGEM7zf(+)
MrpE T113A
MrpE T113Y
MrpE P114G
MrpE P114A
MrpF P28G
MrpF D32A
MrpF R33A
MrpG P81G
kDa
MrpA 75 50
MrpB 20 15
MrpC 20 15
MrpD 50 37
MrpE 20 15
MrpF 15 10
MrpG 20 15
mrpTFCHS7
pGEM7zf(+)
MrpB H34A
MrpB P37G
MrpB F41A
MrpC Q70A
MrpC T75A
MrpC G82P
MrpC G82I
kDa
MrpA 75 50
MrpB 20 15
MrpC 20 15
MrpD 50 37
MrpE 20 15
MrpF 15 10
MrpG 20 15
mrpTFCHS7
pGEM7zf(+)
MrpA H230A
MrpA H230K
pGEM7zf(+)
MrpA H700A
MrpA H700K
MrpA H700W
mrpTFCHS7
pGEM7zf(+)
MrpD K219A
kDa
MrpA 75 50
MrpB 20 15
MrpC 20 15
MrpD 50 37
MrpE 20 15
MrpF 15 10
MrpG 20 15
mrpTFCHS7
pGEM7zf(+)
MrpD F135A
MrpD F136A
MrpD F136G
MrpD F136T
MrpD F136E
MrpD W228A
kDa
MrpA 75 50
MrpB 20 15
MrpC 20 15
MrpD 50 37
MrpE 20 15
MrpF 15 10
MrpG 20 15
mrpTFCHS7
pGEM7zf(+)
MrpD E137D
MrpD E137A
mrpTFCHS7
pGEM7zf(+)
MrpG P81A
MrpG P81G
kDa
MrpA 75 50
MrpB 20 15
MrpC 20 15
MrpD 50 37
MrpE 20 15
MrpF 15 10
MrpG 20 15

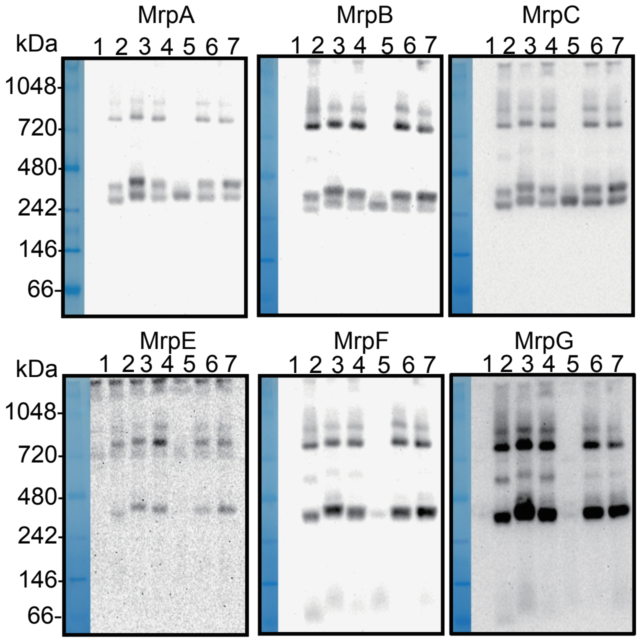
Figure S5



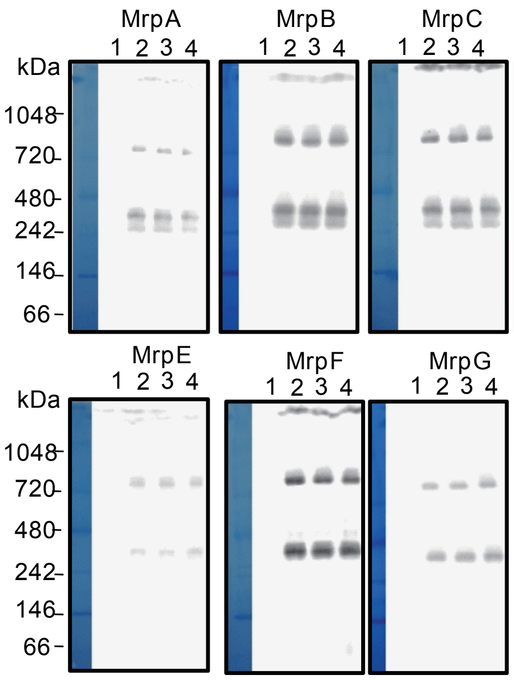
Lane 1: pGEM7zf(+)
Lane 2: mrpTFCHS7
Lane 3: MrpA E140A
Lane 4: MrpA K223A
Lane 5: MrpA K299A
Lane 6: MrpA G392R
Lane 7: MrpA H700A



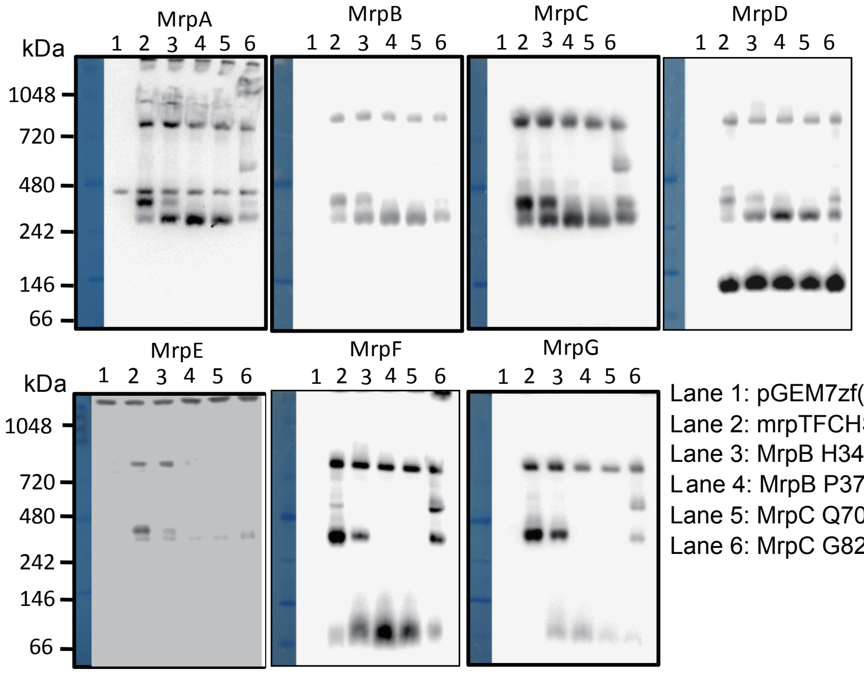
Lane 1: pGEM7zf(+)
Lane 2: mrpTFCHS7
Lane 3: MrpB F41A
Lane 4: MrpC T75A
Lane 5: MrpC G82I
Lane 6: MrpD F136G
Lane 7: MrpD E137A



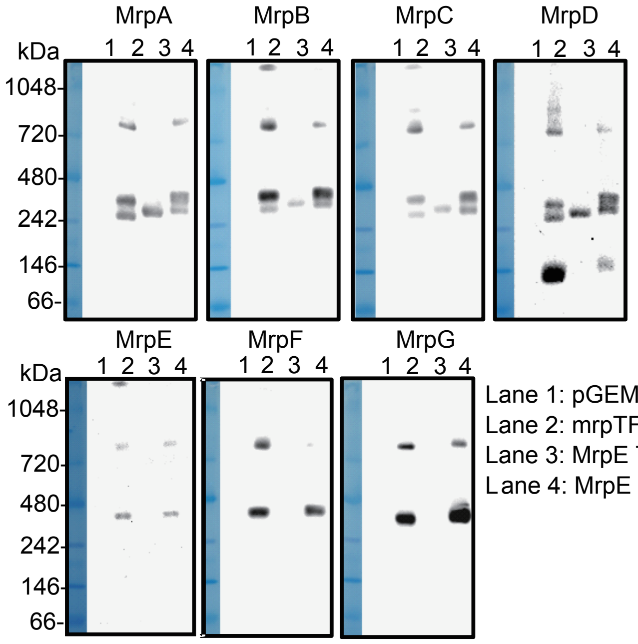
Lane 1: pGEM7zf(+)
Lane 2: mrpTFCHS7
Lane 3: MrpE T113Y
Lane 4: MrpE P114A
Lane 5: MrpF D32A
Lane 6: MrpF R33A
Lane 7: MrpG P81G



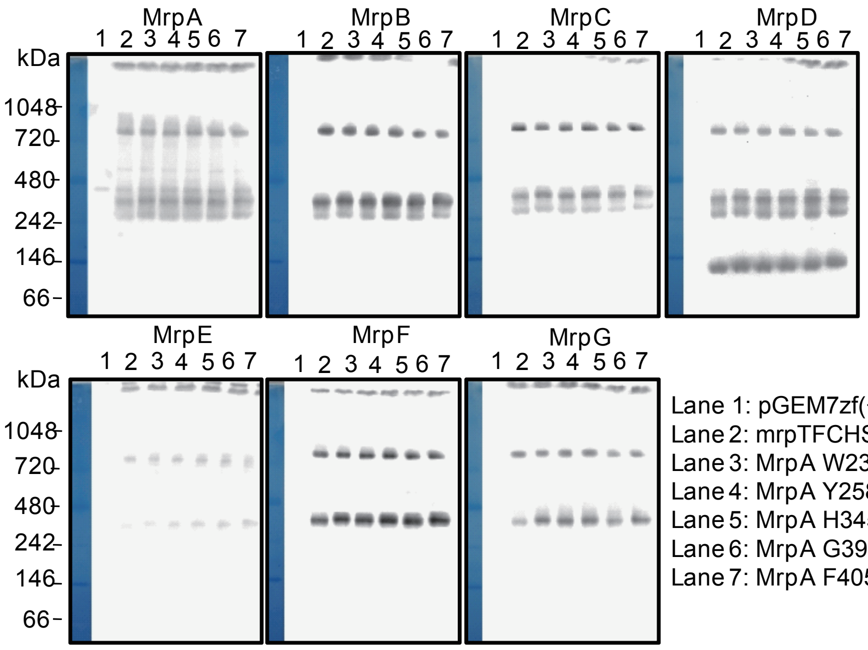
Lane 1: pGEM7zf(+)
Lane 2: mrpTFCHS7
Lane 3: MrpA Y136A
Lane 4: MrpA H230A



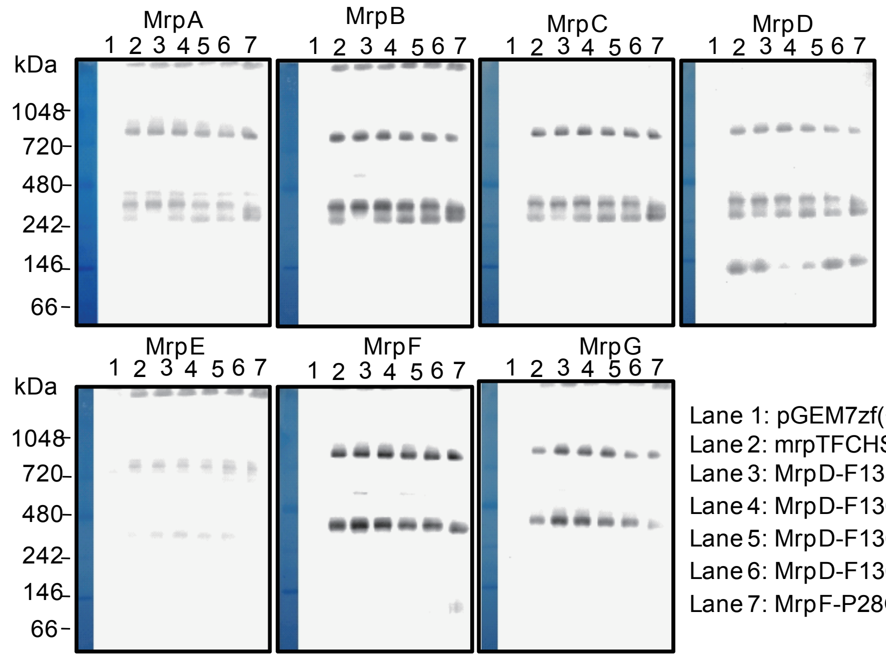
Lane 1: pGEM7zf(+)
Lane 2: mrpTFCHS7
Lane 3: MrpB H34A
Lane 4: MrpB P37G
Lane 5: MrpC Q70A
Lane 6: MrpC G82P



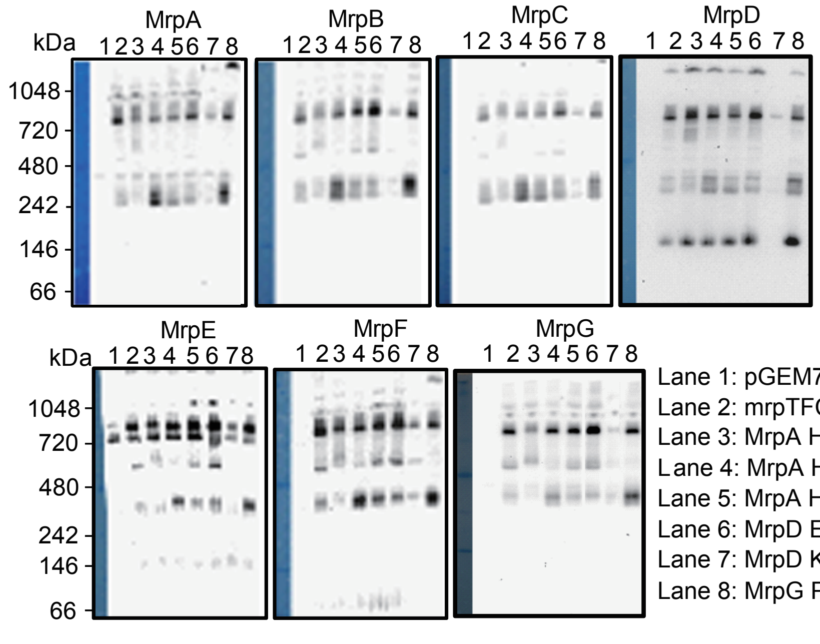
Lane 1: pGEM7zf(+)
Lane 2: mrpTFCHS7
Lane 3: MrpE T113A
Lane 4: MrpE P114G



Lane 1: pGEM7zf(+)
Lane 2: mrpTFCHS7
Lane 3: MrpA W232A
Lane 4: MrpA Y258A
Lane 5: MrpA H345A
Lane 6: MrpA G392A
Lane 7: MrpA F405A



Lane 1: pGEM7zf(+)
Lane 2: mrpTFCHS7
Lane 3: MrpD-F135A
Lane 4: MrpD-F136A
Lane 5: MrpD-F136T
Lane 6: MrpD-F136E
Lane 7: MrpF-P28G



Lane 1: pGEM7zf(+)
Lane 2: mrpTFCHS7
Lane 3: MrpA H230K
Lane 4: MrpA H700K
Lane 5: MrpA H700W
Lane 6: MrpD E137D
Lane 7: MrpD K219A
Lane 8: MrpG P81A

Figure S6