Supplementary Table 1. Primers for cloning *mrpJ* homologs

Name	Sequence[a]
mrpJ'-5'	5'-TA<u>CCATGG</u>TGAAATATCGTCGTGC-3'
mrpJ'-3'	5'-TA<u>AAGCTT</u>AATACAGAAATGCGTC-3'
fimbria2-5'	5'-GA<u>CCATGG</u>AAAGAGAAAAATACG-3'
fim2J3'Bam	5'-TA<u>GGATCC</u>AATTAAAAGCTAATCATCAG-3'
ucaJ-5'	5'-T<u>CCATGG</u>AGAATGAAGTTCATGAG-3'
ucaJ3'Hind	5'-CT<u>AAGCTT</u>ATGTGGGATACATGTCG -3'
fimbria5-5'	5'-GA<u>CCATGG</u>AATGCTTTCACAAATG-3'
fim5J3'Hind	5'-TC<u>AAGCTT</u>AATACCTATCAGTTAATTC-3'
fim8J-5'all	5'-AT<u>CCATGG</u>TTGAGGAAGTGAATTTTGG-3'
fim8J3'Hind	5'-AT<u>AAGCTT</u>GCCACCAATAATATTAT-3'
fim10J1-5'all	5'-CA<u>CCATGG</u>AGCCATCTTGAATTTACTC-3'
fim10J1-3'Hind	5'-AG<u>AAGCTT</u>GAGAAAAACAATAATGTAGC-3'
fimbria10J2-5'	5'-AA<u>CCATGG</u>TTATATGAGTGATAATAATAT-3'
fim10J2-3'Hind	5'-AT<u>AAGCTT</u>AACTAGCGCCAAAGTTCAC-3'
pmpJ-5'	5'-GA<u>CCATGG</u>CTTTTCATGAATATCGATAA-3'
pmpJ3'Hind	5'-GA<u>AAGCTT</u>AAATCATCTAAACACGC-3'
atfJ-5'	5'-GC<u>CCATGG</u>AAATGATTGATAGCAGCAAAAC-3'
atfJ3'Hind	5'- AT<u>AAGCTT</u>AAGGGCGATTAGTGCCA -3'
fim14J-5'	5'-TC<u>CCATGG</u>AGTGTAATTAATGAAATC-3'
fim14J-3'	5'-AT<u>AAGCTT</u>AAAAGTTATTTATGAAG-3'
PMI0182-5'	5'-GA<u>CCATGG</u>TCACCAATGTCAAAAGTT-3'

PMI0182-3'   5'-AG<u>AAGCTT</u>AAATTTGACTCTGTGCGCTCC-3'

PMI0982-5'   5'-TT<u>CCATGG</u>ATGATCATGATAAACTCCAC-3'

PMI0982-3'   5'-AT<u>AAGCTT</u>GGCATTATTTTTCTTTTATTGG-3'

PMI1817-5'   5'-AG<u>CCATGG</u>CGAGAACATATTATGAG-3'

PMI1817-3'   5'-AA<u>AAGCTT</u>ACAATCAGAAAATAG-3'

PMI3508-5'   5'-TT<u>CCATGG</u>CGTATATGATGATCAATAC-3'

PMI3508-3'   5'-TT<u>AAGCTT</u>GCACTATTGTGATAATATTA-3'

[a]Restriction enzyme sites used for cloning are underlined.

Supplementary Table 2. Primers for site-directed mutagenesis of *mrpJ*.

Name	Sequence[a]
S44A 5'	AAAATTGGTGTCGCCCAGCAACAGTTTTCTCGC
S44A 3'	GCGAGAAAACTGTTGCTGGGCGACACCAATTTT
Q45A 5'	AAAATTGGTGTCAGCGCGCAACAGTTTTCTCGCT
Q45A 3'	AGCGAGAAAACTGTTGCGCGCTGACACCAATTTT
Q46A 5'	ATTGGTGTCAGCCAGGCACAGTTTTCTCGCTATG
Q46A 3'	CATAGCGAGAAAACTGTGCCTGGCTGACACCAAT
Q47A 5'	GGTGTCAGCCAGCAAGCGTTTTCTCGCTATGAACG
Q47A 3'	CGTTCATAGCGAGAAAACGCTTGCTGGCTGACACC
F48A 5'	CAGCCAGCAACAGGCTTCTCGCTATGAACGAGG
F48A 3'	CCTCGTTCATAGCGAGAAGCCTGTTGCTGGCTG
S49A 5'	CAGCCAGCAACAGTTTGCTCGCTATGAACGAGG
S49A 3'	CCTCGTTCATAGCGAGCAAACTGTTGCTGGCTG
R50A 5'	GCCAGCAACAGTTTTCTGCCTATGAACGAGGTATG
R50A 3'	CATACCTCGTTCATAGGCAGAAAACTGTTGCTGGC
Y51A 5'	CAGCAACAGTTTTCTCGCGCTGAACGAGGTATGAAC
Y51A 3'	GTTCATACCTCGTTCAGCGCGAGAAAACTGTTGCTG
E52A 5'	GCAACAGTTTTCTCGCTATGCACGAGGTATGAACAA
E52A 3'	TTGTTCATACCTCGTGCATAGCGAGAAAACTGTTGC
G20D 5'	GTAAATGCTTCTGTAGATAAAAGATCCAAAAAAAACG
G20D 3'	CGTTTTTTTTGGATCTTTTTATCTACAGAAGCATTTAC
I23D 5'	CTGTAGGTAAAAGGACCAAAAAAAACGTAAAGAGCTGGG

I23D 3'   CCCAGCTCTTTACGTTTTTTTTGG<u>TC</u>CTTTTTACCTACAG

R27D 5'   GATCCAAAAAAAA<u>GA</u>TAAAGAGCTGGGTTATACCGG

R27D 3'   CCGGTATAACCCAGCTCTTTA<u>TC</u>TTTTTTTTGGATC

T33D 5'   CGTAAAGAGCTGGGTTAT<u>GA</u>CGGTATGCAGCTGGC

T33D 3'   GCCAGCTGCATACCG<u>TC</u>ATAACCCAGCTCTTTACG

G34D 5'   CGTAAAGAGCTGGGTTATACCG<u>A</u>TATGCAGCTGGC

G34D 3'   GCCAGCTGCATA<u>T</u>CGGTATAACCCAGCTCTTTACG

L37D 5'   TACCGGTATGCAG<u>GAT</u>GCTAAAAAAATTGGTGTCAGCC

L37D 3'   GGCTGACACCAATTTTTTTAGC<u>ATC</u>CTGCATACCGGTA

G54D 5'   CTCGCTATGAACGAG<u>A</u>TATGAACAAAATAGATCTC

G54D 3'   GAGATCTATTTTGTTCATA<u>T</u>CTCGTTCATAGCGAG

I58D 5'   GAACGAGGTATGAACAAA<u>GAT</u>GATCTCAGACATTTAGTGTTG

I58D 3'   CAACACTAAATGTCTGAGATC<u>ATC</u>TTTGTTCATACCTCGTTC

L63D 5'   GAACAAAATAGATCTCAGACAT<u>GAT</u>GTGTTGTTAGCTCTC

L63D 3'   GAGAGCTAACAACAC<u>ATC</u>ATGTCTGAGATCTATTTTGTTC

[a]Nucleotides that were changed from *mrpJ* to give the desired amino acid substitution are underlined.

Supplementary Table 3. Primers for *flhDC* and *flaA* analysis.

Primers for amplifying DNA targets used in EMSA

Name	Sequence
flhD5'	5'-ATGAGTACGGTTGAATTGC-3'
flhD3'	5'-TTATGCCCGTTTCTTTGTAGC-3'
flhDpro5'	5'- ATGAGGAGGAGCTATGGC -3'
flhDpro3'	5'- CTCTTTACATCCCGTCCG -3'

qRT-PCR primers

Name	Sequence
rpoA-RT5'3	5'-GCAAATCTGGCATTGGCCCTGTTA-3'
rpoA-RT3'3	5'-TAGGGCGCTCATCTTCTTCCGAAT-3'
flhD-5'	5'-CCGGTTTGAAGACAGCGAAACA-3'
flhD-3'	5'-TGCCCGTTTCTTTGTAGCAGAGGT-3'
flaA-5'	5'-GGTGCTGCAATCGATGCGAAAGAT-3'
flaA-3'	5'-TGTCAGCACCTTCCAGTGCGAAAT-3'

N   C

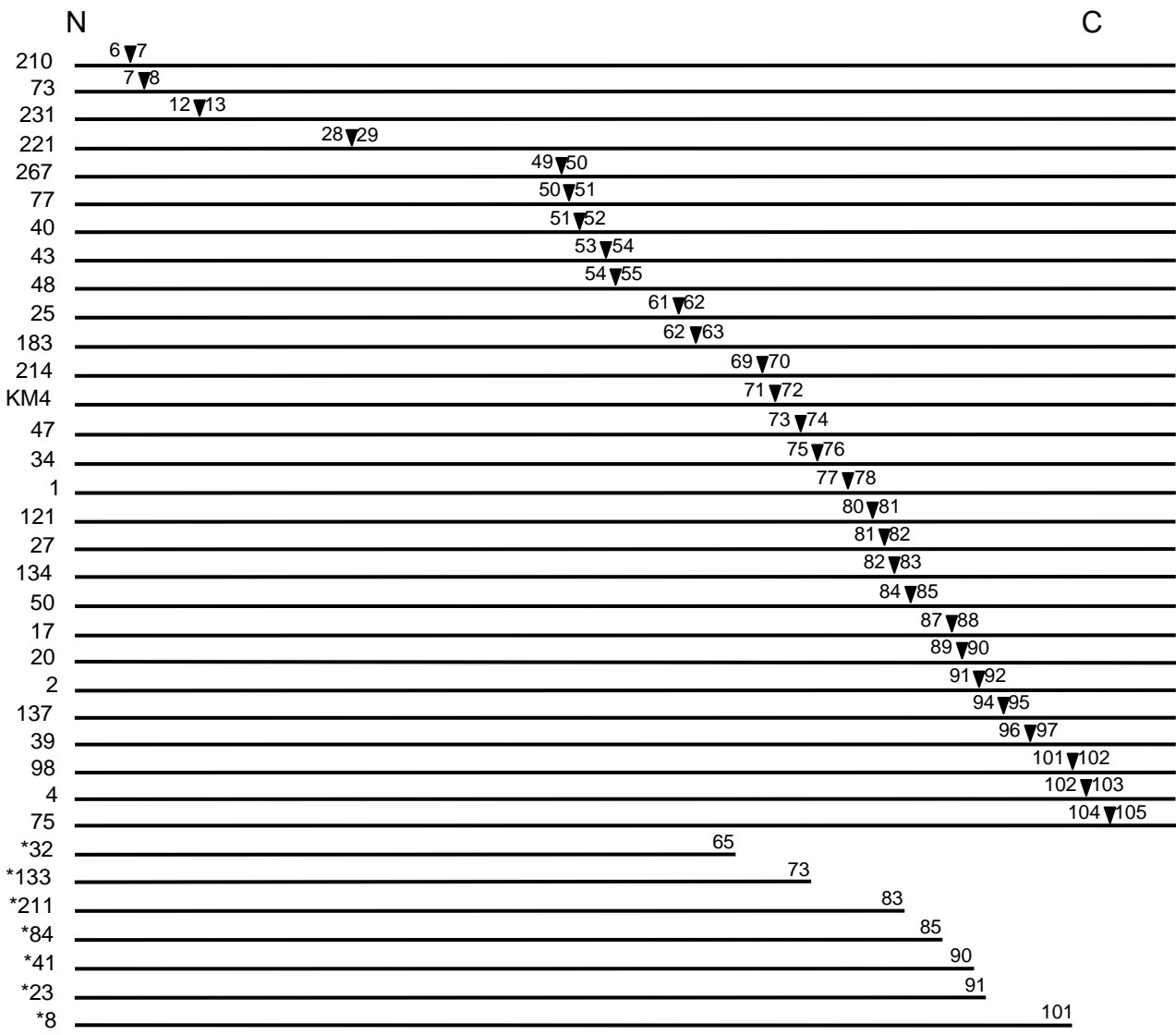| Mutant	Insertion site
210	6 ▼7
73	7 ▼8
231	12 ▼13
221	28 ▼29
267	49 ▼50
77	50 ▼51
40	51 ▼52
43	53 ▼54
48	54 ▼55
25	61 ▼62
183	62 ▼63
214	69 ▼70
KM4	71 ▼72
47	73 ▼74
34	75 ▼76
1	77 ▼78
121	80 ▼81
27	81 ▼82
134	82 ▼83
50	84 ▼85
17	87 ▼88
20	89 ▼90
2	91 ▼92
137	94 ▼95
39	96 ▼97
98	101 ▼102
4	102 ▼103
75	104 ▼105
*32	65
*133	73
*211	83
*84	85
*41	90
*23	91
*8	101

Fig S1. Schematic of the amino acid sequence of MrpJ with the position of random 5-aa insertions indicated with inverted triangles. The numbers flanking each triangle refer to the amino acids flanking the insertion site. The numbers on the left denote the name of each mutant. The last seven mutants, indicated with asterisks, have insertions that resulted in premature stop codons.