

Supplementary Materials for

Reconstitution of *Spiroplasma* swimming by expressing two bacterial actins in synthetic minimal bacterium

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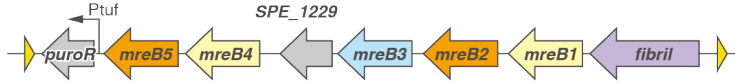
Figs. S1 to S5
Tables S1 to S2
Captions for Movies S1 to S8
Captions for Data S1

Other Supplementary Materials for this manuscript include the following:

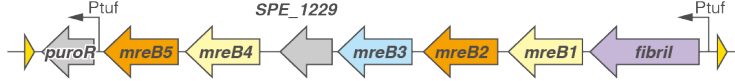
Movies S1 to S8
Data S1

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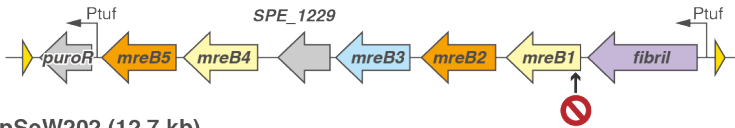
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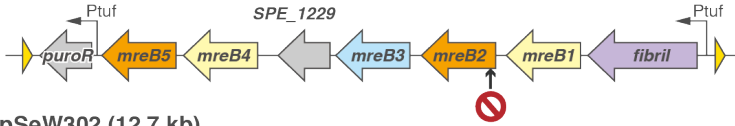
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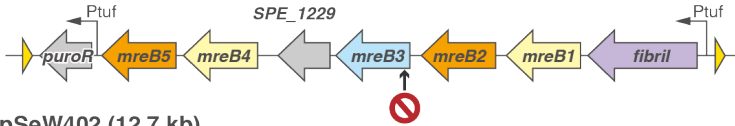
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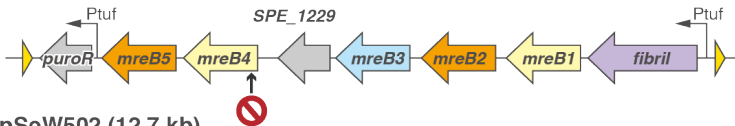
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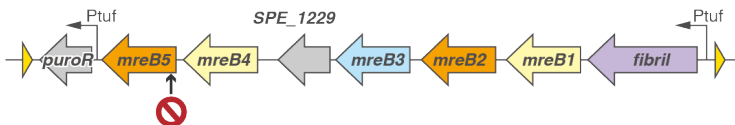
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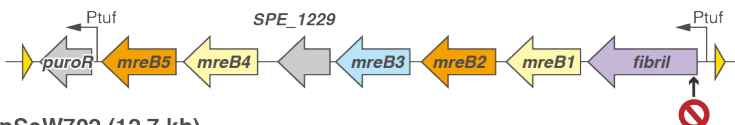
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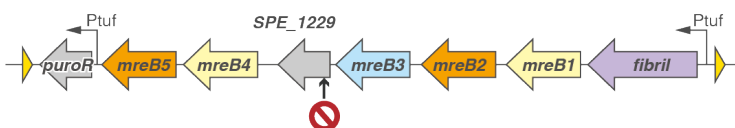
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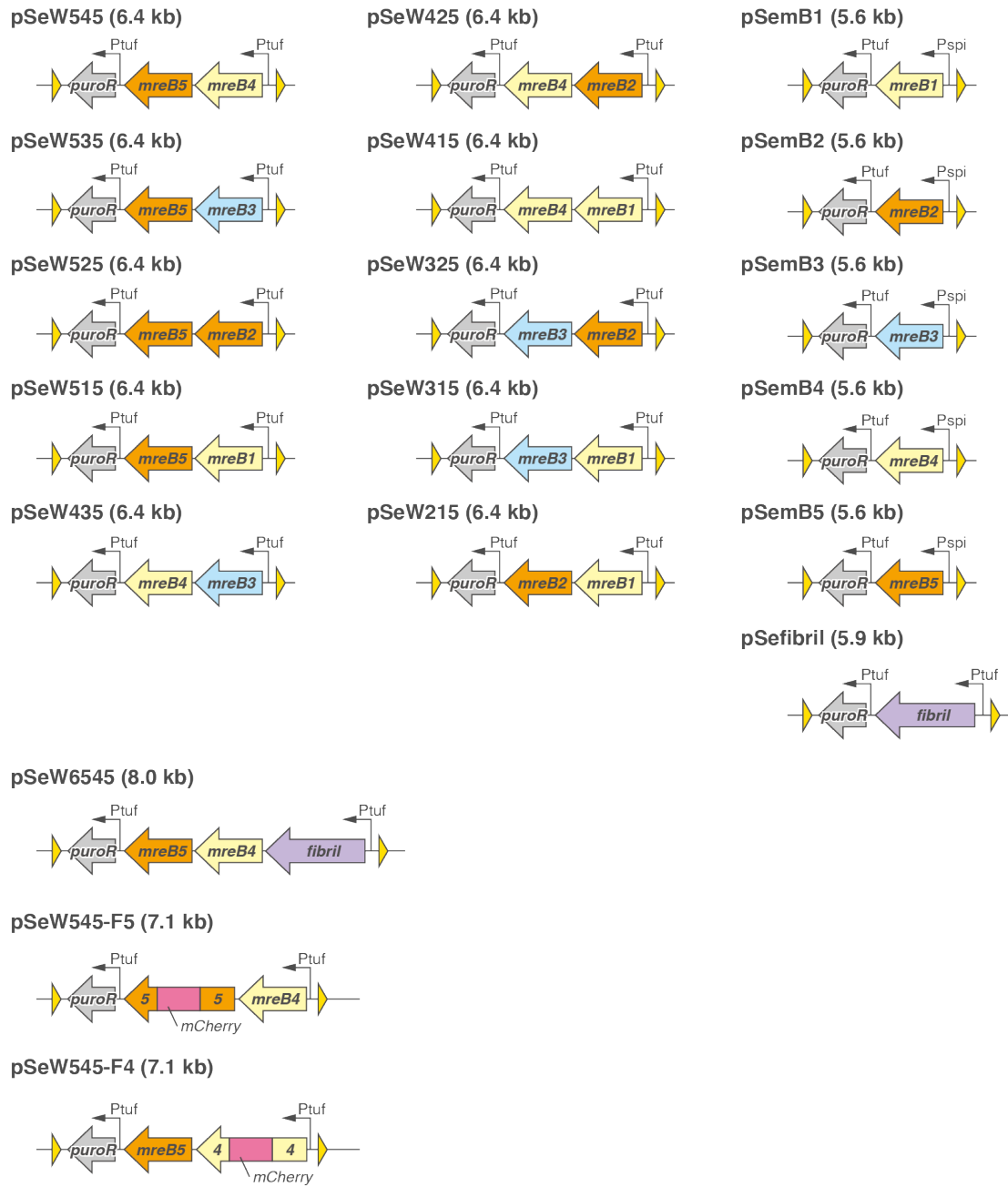


Fig. S1.
Schematic presentation for DNA constructs used in this study.

S. eriocheiris

syn3Bsw

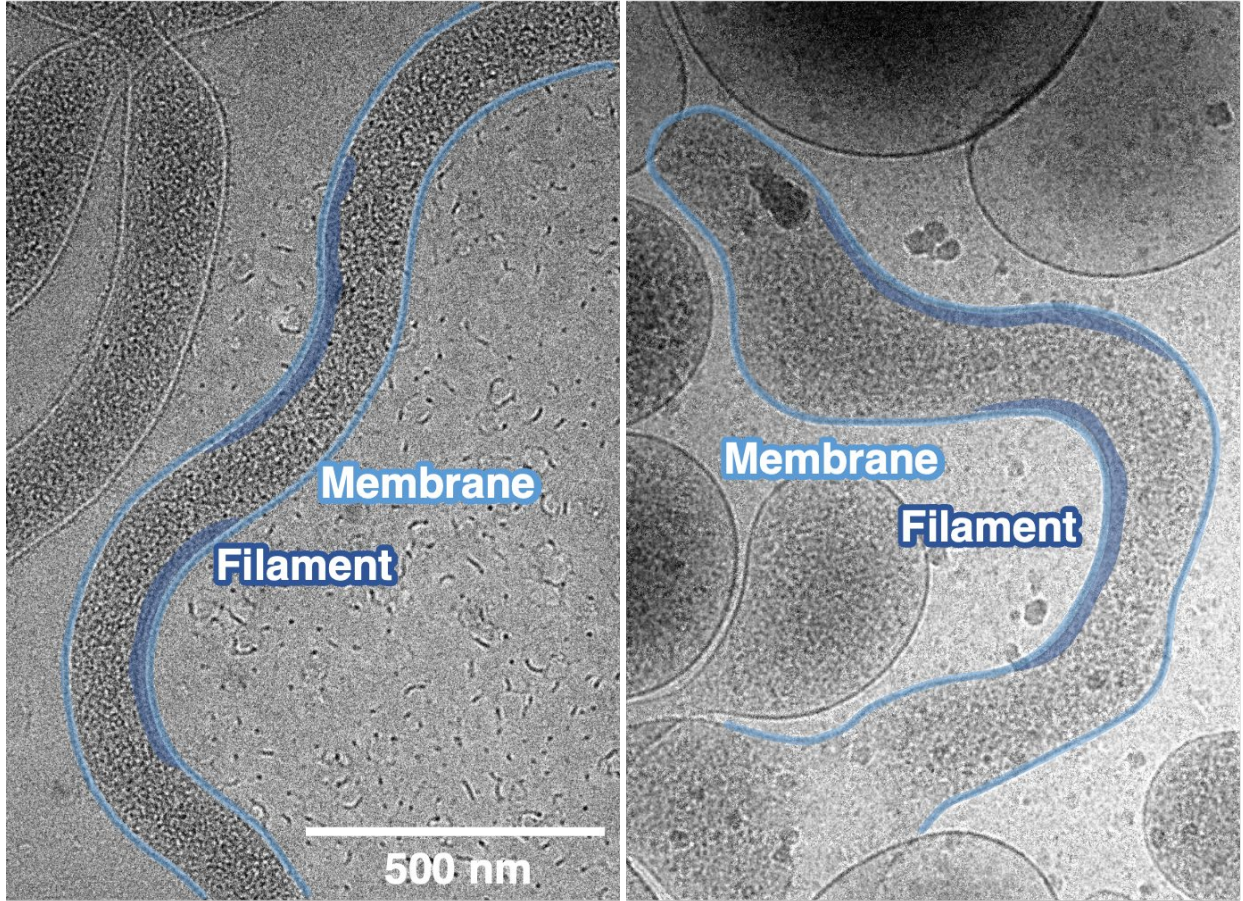


Fig. S2.

Cell images under cryo electron microscopy. The cell membrane and filamentous structures are colored light and dark blue, respectively.

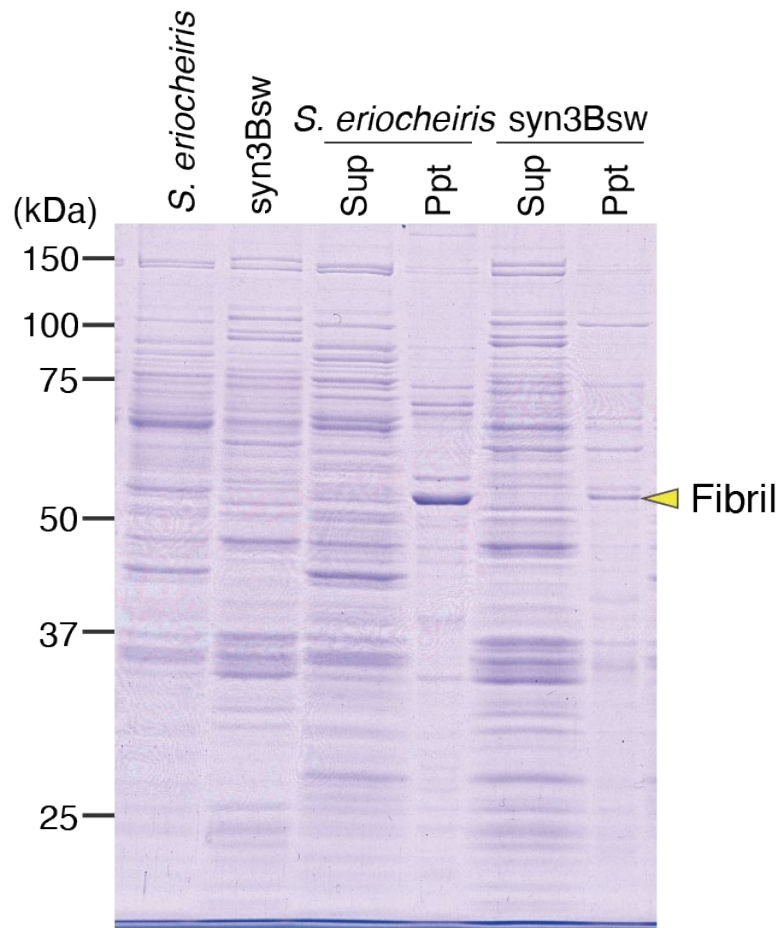


Fig. S3.

Identification of fibril protein in *syn3Bsw*. The cultured cells were treated by *** and fractionated by centrifugation, and analyzed by SDS-10% PAGE. The whole cell lysates are shown in left two lanes. Fibril protein is marked. Protein bands were stained by Coomassie Brilliant Blue.

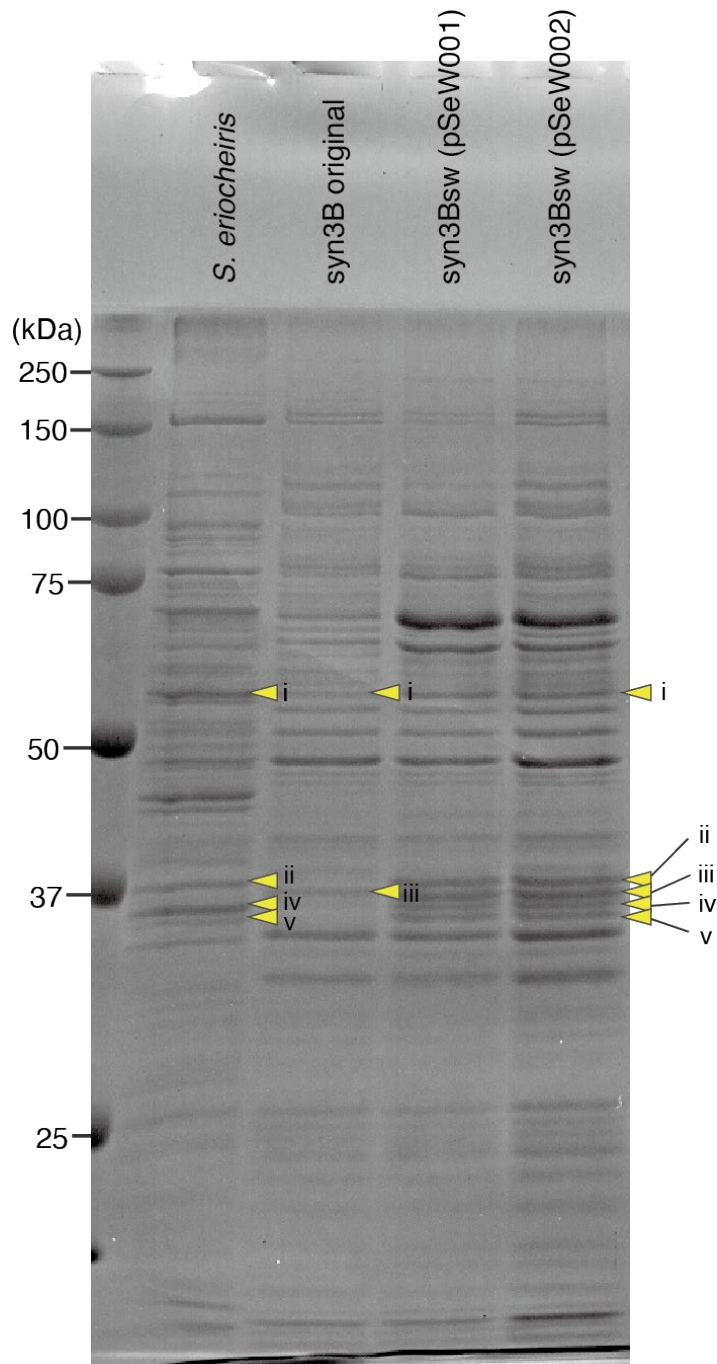


Figure S4.

Whole protein profiles of cell lysates visualized by SDS-12.5% PAGE. Constructs are indicated on the top and focused protein bands are marked by a yellow triangle with a number. Protein bands were stained by Coomassie Brilliant Blue.

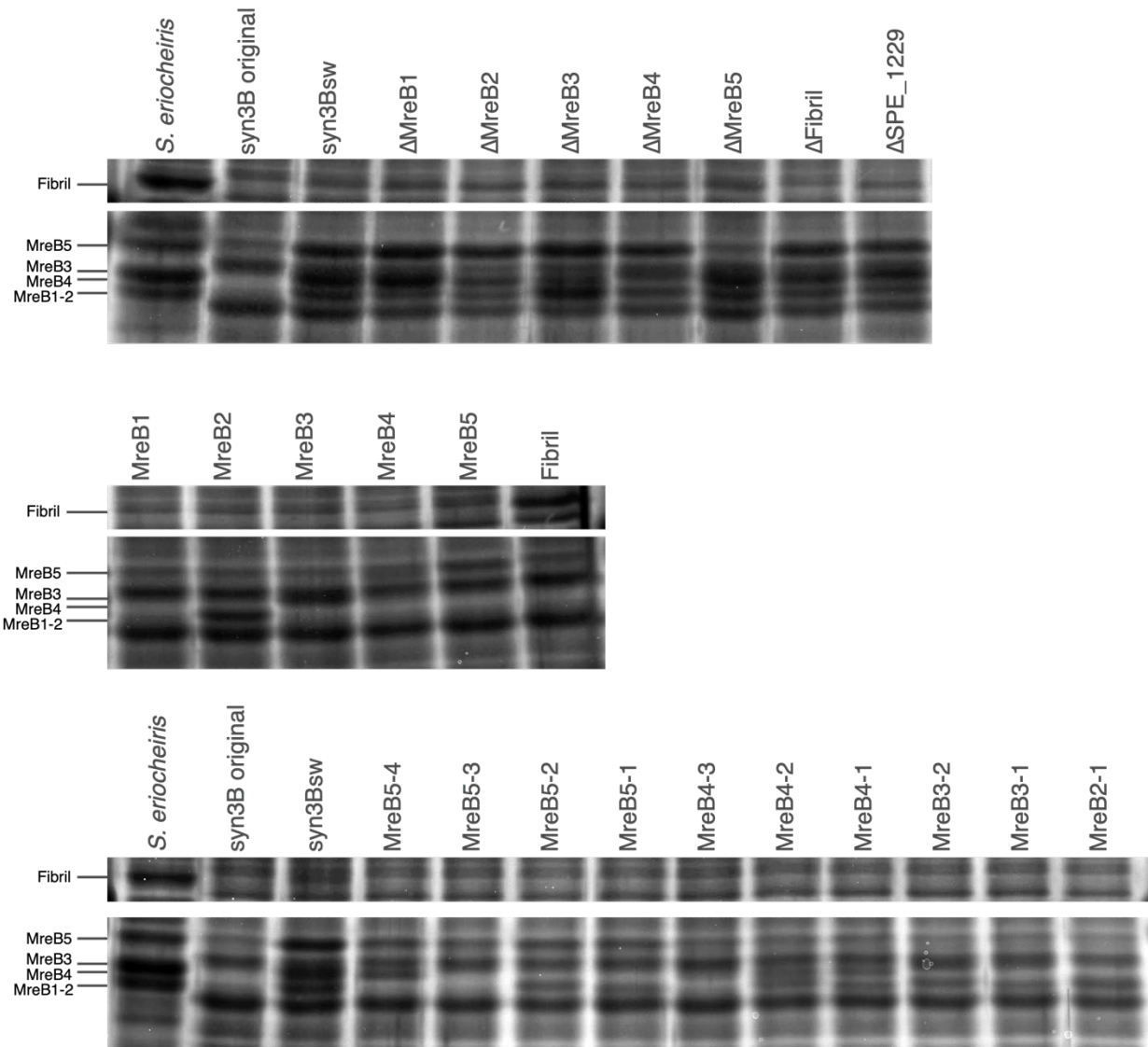


Figure S5.

Profiles of focused proteins visualized by SDS-10% PAGE. Constructs are indicated on the top and the protein names are shown on the left. Protein bands were stained by the reverse staining method.

Table S1.**DNA primers used in this study.**

Name	Sequence	Purpose
w_mreb3to5_R	TATAAAATTTAAAAATTACTTGGTATTGATAGTAAG	pSeW001
w_mreb2_F	ACCAAGTAATTTTTAAATTTTATATTCTTTTGCTTC	pSeW001
w_mreb2_R	TTAGATTATTAATTCGCCCTACTAAAAGTAGTTG	pSeW001
w_mreb1_F	GTGAGGGAGAATTTAATAATCTAATTCTTTGTTTT	pSeW001
w_mreb1_R	AAAAGTGAATAATTGCCTGAAGATTTAATAATAATT	pSeW001
w_fibril_F	ATCTTCAGGCAATTATTCACCTTTTAAACGAATAGT	pSeW001
w_fibril_R	TTGACAGCTAGCTCAGTCCTACCTTAAGTTTACTGGATTTTAAAG	pSeW001
LP_inverse_R	GAACTATATAATAACTCGCATATTG	pSeW001, pSemB1, pSemB2, pSemB3, pSemB4, pSemB5, pSefibril, pSeW435, pSeW425, pSeW415, pSeW325, pSeW315, pSeW215
LP_inverse_F	AGGACTGAGCTAGCTGTCAAAGATC	pSeW001, pSemB2, pSefibril
w_mreb3to5_F	GCGAGTTATTTATATAGTTCTTATTTTTCTTCCCAATGCCAGC	pSeW001, pSemB5
addFib-to079-F	GAGTTATTTATATAGTTCTTATTCACCTTTTAAACGAATAGT	pSeW002, pSefibril
addFib-toPtuf-R	TTTTAAGGAGAAAAAACATGATTGGAGTTATTTCAACTGCG	pSeW002, pSefibril
Ptuf-to079-R	GACAGCTAGCTCAGTCCTTATTTTTGAATTAAGTATTAAAT	pSeW002, pSefibril
Ptuf-F	GTTTTTTCTCCTTAAAATTTCTATAAC	pSeW002, pSefibril, pSeW545, pSeW535, pSeW525, pSeW515, pSeW435, pSeW425, pSeW415, pSeW325, pSeW315, pSeW215
Stop-mreB1-F	TACAAAAGTCGGTACTTATTGTTAATC	pSeW102
Stop-mreB1-R	CAATAAGTAACCGACTTTTGTATCAATT	pSeW102
Stop-mreB2-F	TACGCGACAGTTTATGCAGTTCCTAA	pSeW202
Stop-mreB2-R	GAAGTGCATAAACTGTCGCGTATGTC	pSeW202
Stop-mreB3-F	ACGAGGTGGTTAAGGAGAAATATTAATG	pSeW302
Stop-mreB3-R	TTTCTCCTTAACCACCTCGTAAATTTATTG	pSeW302
stop-mreB4-F	GAAAGTTGGTTATTTACTTTTGCCGCTA	pSeW402
stop-mreB4-R	AAAGTAAATAACCAACTTTCGTTTCAAT	pSeW402
Stop-mreB5-F2	CCCTTGACCTTAAACGTAAGCTAACACG	pSeW502
Stop-mreB5-R2	CTTACGTTTAAGGTCAAGGGATTATCTA	pSeW502
MreB1-F	GCGAGTTATTTATATAGTTCTTAATAATCTAATTCTTTGTTTT	pSemB1
MreB1-Pspi-R	GAGAAAGGAAATATAAGATCATGGCATTGATTAACAATAAGAAA	pSemB1
Pspi_inverse-F	GATCTTATATTTCTTTCTCTATT	pSemB1, pSemB3, pSemB4, pSemB5
MreB2_R	ATGGCTAATTATAAATTTGGAAAA	pSemB2
MreB2_Pspi_F	AAATTTATAATTAGCCATGATCTTATATTTCTTTCTCTATTAAGTAG	pSemB2
LP_insert_Pspi_R	GACAGCTAGCTCAGTCCTAATTAAGTTAGTGAACAAGAAA	pSemB2
LP_insert_MreB2_F	GAGTTATTTATATAGTTCTTAAATTTTATATTCTTTTGCTTC	pSemB2, pSeW215
MreB3-Pspi-R	GAGAAAGGAAATATAAGATCATGACTATAACAGACGTATTAAAA	pSemB3
MreB3-F	GCGAGTTATTTATATAGTTCTTATTTATTTTTTTTATTTTCTTC	pSemB3, pSeW325, pSeW315
MreB4-Pspi-R	GAGAAAGGAAATATAAGATCATGTTAGATATTGTTTATGTTTAT	pSemB4
MreB4-F	GCGAGTTATTTATATAGTTCTTAGTAATCTAATTCTTTAGTATG	pSemB4, pSeW435, pSeW425, pSeW415
MreB5-Pspi-R	GAGAAAGGAAATATAAGATCGTGAACAGAAAGACCATTTATC	pSemB5
mreB4-Ptuf-ORF-R	AATTTTAAGGAGAAAAAACATGGCAGGATTTAATAGCGGCAA	pSeW545
SD-mreB5-R	ATATTAAGGAGGAAATTAACGTGAA	pSeW535, pSeW525, pSeW515
mreB3-Ptuf-R	AATTTTAAGGAGAAAAAACATGACTATAACAGACGTATTAAAA	pSeW535, pSeW435
mreB3-SD-mreB5-F	GTTAATTTCTCCTTAATATTTATTTATTTTTTTTATTTTCTTCAATT	pSeW535
mreB2- Ptuf-R	AATTTTAAGGAGAAAAAACATGGCTAATTATAAATTTGGAAAA	pSeW525, pSeW425, pSeW325

mreB2- SD-mreB5-F	GTTAATTCCTCCTTAATATTTAAATTTTATATTCTTTTGCTTC	pSeW525
mreB1- Ptuf-R	AATTTTAAGGAGAAAAAACATGGCATTGATTAACAATAAGAAA	pSeW515, pSeW415, pSeW315, pSeW215
mreB1- SD-mreB5-F	GTTAATTCCTCCTTAATATTTAATAATCTAATTCTTTGTTTTTG	pSeW515
mreB3-mreB4-F	CCTCCCTTTTTTATTTATTTTTTTTATTTTCTTC	pSeW435
mreB4-mreB3-R	AAATAAATAAAAAAGGGAGGAATTTTACAATG	pSeW435
mreB2_mreB4_F	CCTCCCTTTTTTAAATTTTATATTCTTTTGCTTC	pSeW425
mreB2_mreB4_R	TAAAAATTTAAAAAAGGGAGGAATTTTACAAT	pSeW425
mreB1-only-F	TTAATAATCTAATTCTTTGTTTTTGA	pSeW415, pSeW315
mreB4-mreB1-R	ACAAAGAATTAGATTATTAATAAAGGGAGGAATTTTACAATG	pSeW415
mreB3-mreB1-R	ACAAAGAATTAGATTATTAATGAAAGGAGACAATCATAGATG	pSeW315
mreB4-mChe-F	ATAATAGCTGATGATCCTGAGTATTTTGCTAATGAACCAATTC	pSeW545-F4
mreB4-mChe-R	ATAAAAGTGGAGCTCCTGGTCCAGACGAAAGAAAAATGAAAGTT	pSeW545-F4
mreB5-mChe-F	ATAATAGCTGATGATCCTGAGTATTTAACTAATGAACCGATGTA	pSeW545-F5
mreB5-mChe-R	ATAAAAGTGGAGCTCCTGGTCATAATGAACGTGCAATGCAAATT	pSeW545-F5
SWmCh-linker-R2	TCAGGATCATCAGCTATTATTAAGAATTT	pSeW545-F5, pSeW545-F4
SWmCh-linker-F	ACCAGGAGCTCCACTTTTATATAGTTTCATCCATACCAC	pSeW545-F5, pSeW545-F4
mreB4-SD-fibril-R	TTCGTTTTAAAAAGTGAATAAAAAAGGGAGGAATTTTACAATGG	pSeW6545
fibril-F	TTATTCACTTTTTAAACGAATAGTTAC	pSeW6545
coloP-puroR-F-Hana	ggagtagtccaacagcaacagca	colony PCR
syn3B-junc-F	TATGTGATAATGCCAATCGCTAAG	colonyPCR
syn3B-junc-R	GTAAATTCCTCAAATTTCCATCA	colonyPCR

Table S2.

Protein identification by mass spectrometry for Fig. S4.

Bacterial strain	Protein band	Gene ID	Annotation	Mass (kDa)	score	coverage (%)
<i>S. eriocheiris</i>	i	SPE_0666	Fibril	58.7	184	63
<i>S. eriocheiris</i>	ii	SPE_1231	MreB5	38.7	117	54
<i>S. eriocheiris</i>	iv	SPE_1230	MreB4	40.7	64	49
<i>S. eriocheiris</i>	iv	SPE_1049	GAPDH	35.8	129	68
<i>S. eriocheiris</i>	v	SPE_1224	MreB2	37.9	55	36
original syn3B	i	ODP2_MYCCT	2-oxo acid dehydrogenase subunit E2	47	43	24
original syn3B	iii	-	-	-	-	-
syn3Bsw	i	SPE_0666	Fibril	58.7	124	52
syn3Bsw	i	ODP2_MYCCT	2-oxo acid dehydrogenase subunit E2	47	69	36
syn3Bsw	ii	SPE_1231	MreB5	38.7	131	57
syn3Bsw	iii	SPE_1228	MreB3	38.5	187	71
syn3Bsw	iv	SPE_1230	MreB4	40.7	86	57
syn3Bsw	iv	SPE_1228	MreB3	38.5	89	48
syn3Bsw	iv	SPE_1224	MreB2	37.9	46	34
syn3Bsw	v	SPE_1224	MreB2	37.9	101	42
syn3Bsw	v	SPE_0470	MreB1	38	96	45

Movie S1.

Cell behaviors of three strains indicated on top. Real time movie for 5 s.

Movie S2.Rotational behaviors of freely moving site of *Spiroplasma* and syn3Bsw cells for 10 s.**Movie S3.**

Cells lacking one of seven proteins from syn3sw for 10 s.

Movie S4.Cells expressing single *Spiroplasma* protein for 5 s.**Movie S5.**

Syn3B cells expressing pair of SMreBs for 10 s.

Movie S6.

Cells expressing single protein for 10 s.

Movie S7.

SMreB5 localization in cell expressing SMreB 4 and 5 visualized by fluorescence for 10 s.

Movie S8.

syn3B cells expressing SMreBs 4, 5, and fibril for 10 s.

Data S1. (separate file)

DNA sequences of constructs used in this study.