Supplemental material

A tailored galK counterselection system for efficient markerless gene deletion and chromosomal tagging in Magnetospirillum gryphiswaldense

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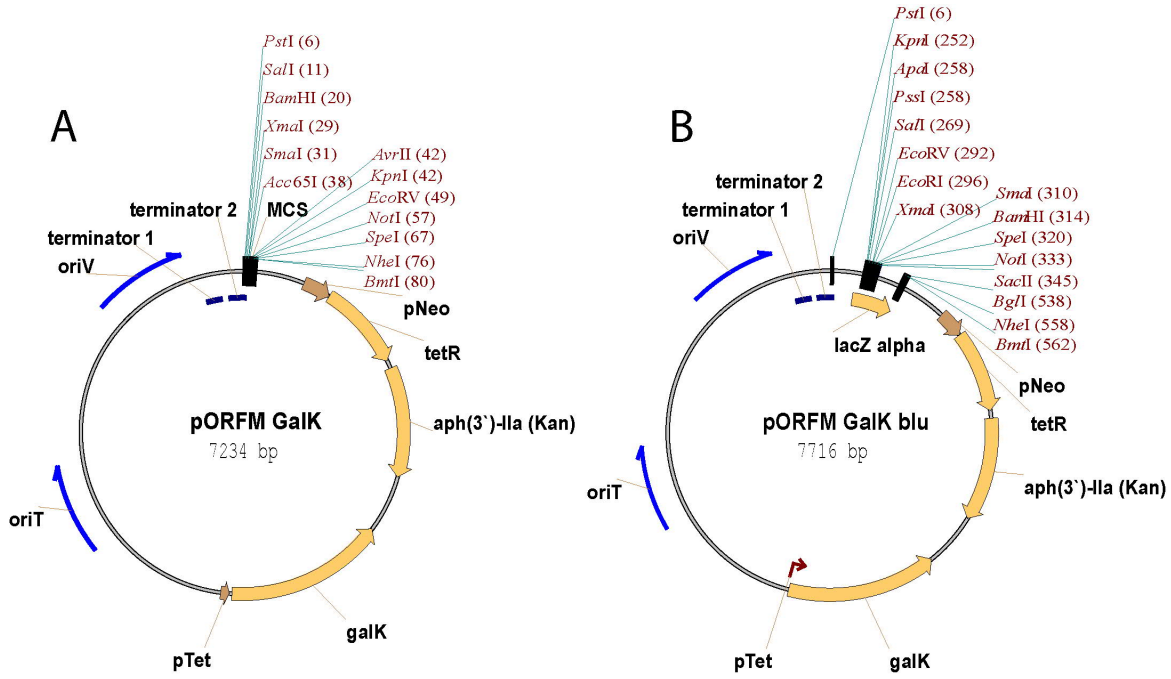

Figure S1. Vector maps of (A) pORFM GalK and (B) pORFM GalK blu. All relevant plasmid features are indicated. Unique restrictions sites located in the multiple coloning site (MCS) or the lacZ gene fragment, respectivly are labelled in red.

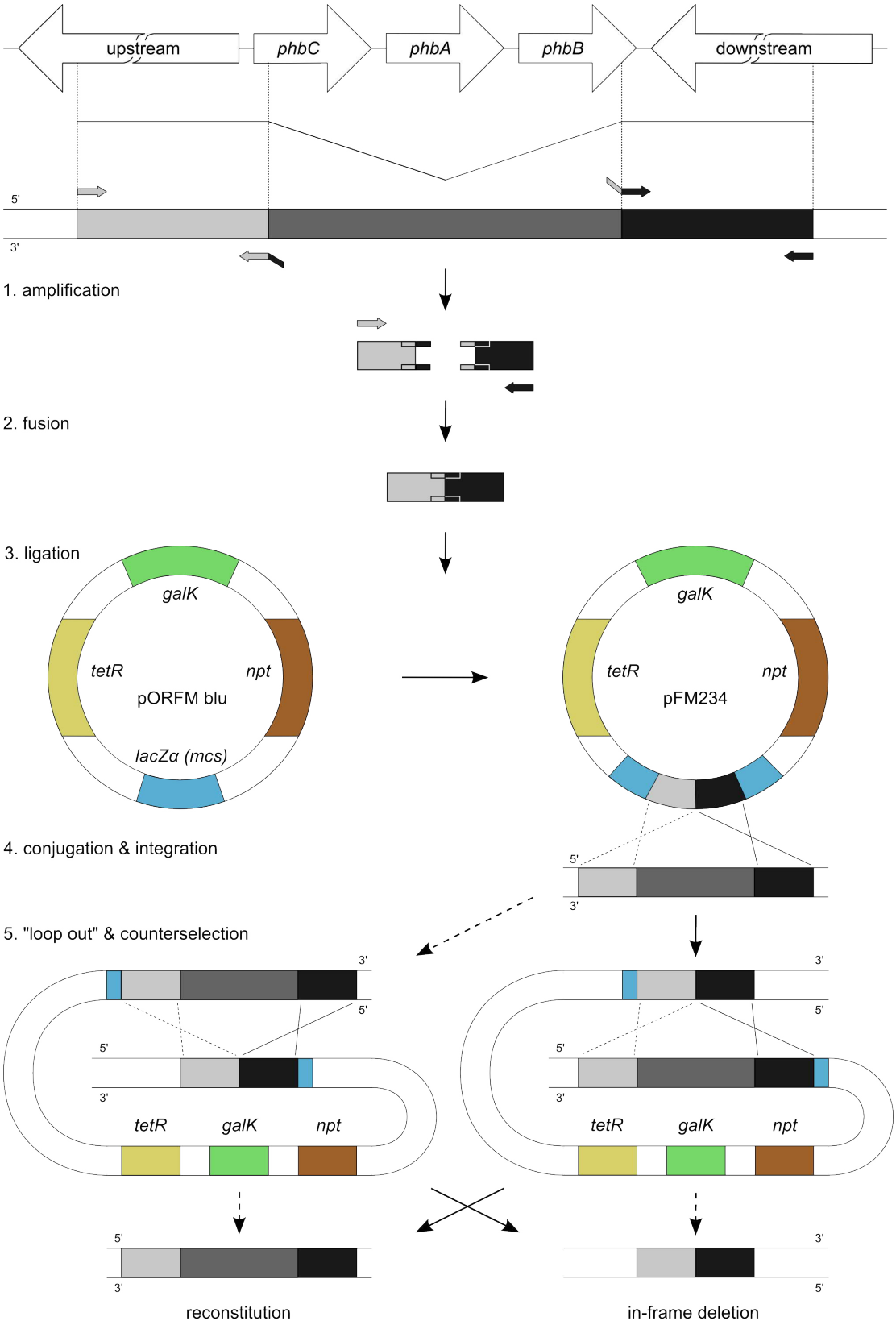
upstream
phbC
phbA
phbB
downstream
5'
3'
1. amplification
2. fusion
3. ligation
galK
tetR
npt
pORFM blu
lacZα (mcs)
pFM234
4. conjugation & integration
5. "loop out" & counterselection
reconstitution
in-frame deletion

Figure S2. Scheme of in-frame gene deletion in MSR-1 with pORFM blu exemplified by the phbCAB operon. First, homologous regions up- and downstream of the target are amplified by PCR and fused by a second, overlap-extension PCR. This generates the mutated in-frame allele *in vitro* where the first and last codons of the target coding region are fused in-frame generating a short nonsense ORF. The PCR product becomes blunt-end ligated into pORFM blu and transformed into *E. coli* DH5α. White, kanamycin resistant clones are selected and the inserts of their plasmids are sequenced with vector specific primers oFM280b and oFM281b to verify absence of mutations within the homologous regions (not shown). Subsequently, the vector is transformed into *E. coli* BW29427 and conjugated into MSR-1. Recombinant, merodiploid clones are isolated from kanamycin supplemented FSM plates and screened for up or downstream integration of the plasmid (step 4, dashed or solid lines, respectively and illustrated in Figure S2 for the fusion of *mamC* to *egfp* and *mCherry*). At least one of each integration type is used for counterselection with galactose which results either in reconstitution of the wt genotype or in-frame deletion of the target sequence (step 5).

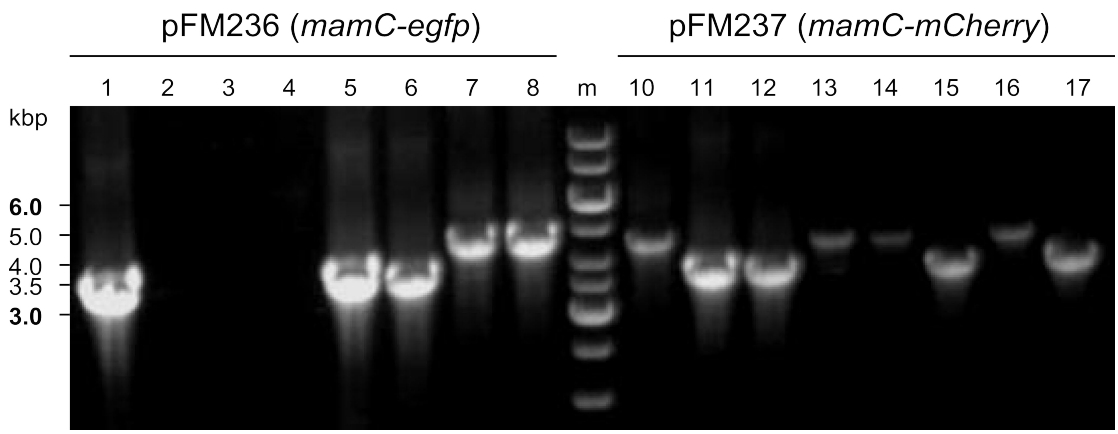

Figure S3. Result of up- or downstream vector insertion diagnosis after pFM236 (mamC-egfp, lanes 1-8) and pFM237 (mamC-mCherry, lanes 10-17) conjugation. Larger fragments represent vector insertions upstream of mamC, smaller fragments represent downstream insertions. The size difference of the DNA fragments corresponds to egfp and mCherry (approximately 720 bp) respectively. m: DNA size standard (marker). Cells were picked from FSM plates with kanamycin and grown in 96-well plates over night prior to colony-PCR with oligonucleotide primers oFM281/oFM289a. One strain of each insertion type was used for counterselection on galactose plates.

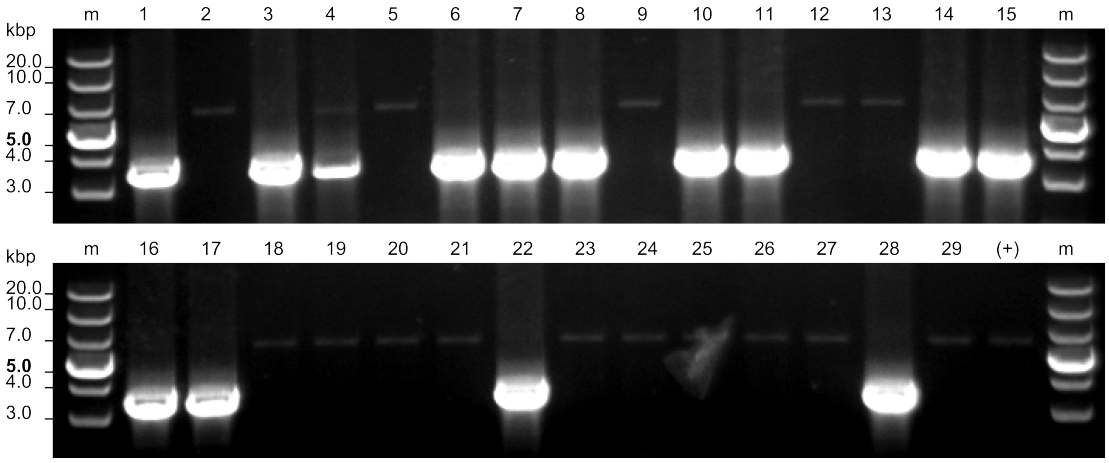

Figure S4. Result of colony-PCR diagnosis for phbCAB deletion after galactose counterselection. Of the 29 colonies tested, 13 likely contain the deletion (as suggest by the lower ~ 3.5 kb band), 15 converted back to wt (~7 kb band) and one was inconclusive (lane 4). (+) = wt control, m = DNA size standard (marker).

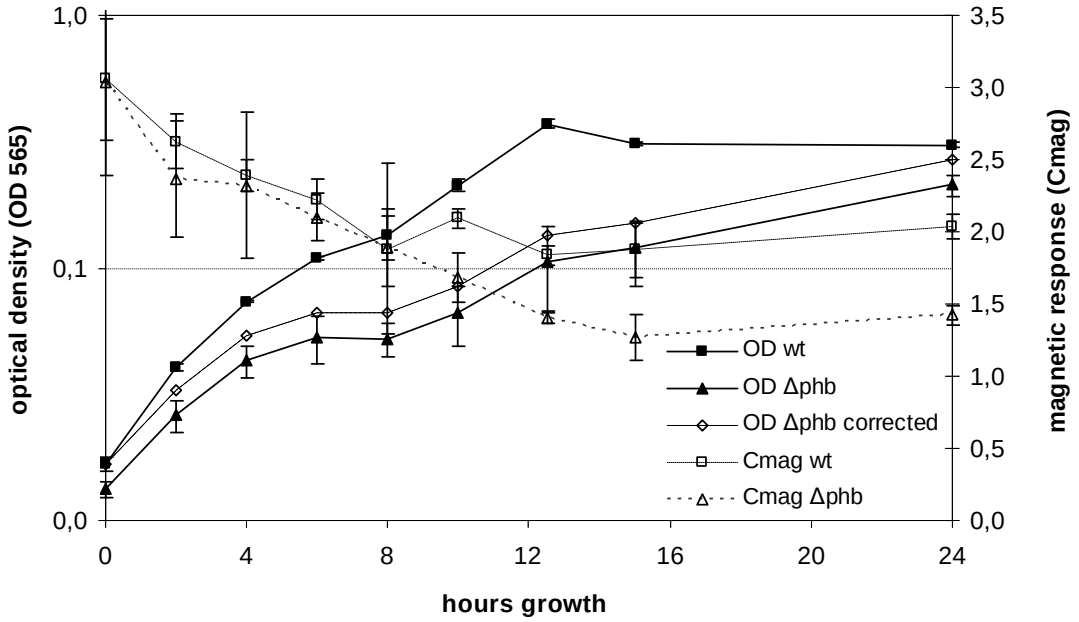

Figure S5. Growth and magnetic response of phbCAB mutant and wt cultures as determined by light scattering and cell counts. OD measurements of the Δ phbCAB mutant (filled triangles) suggest slight growth impairment compared to wt (filled squares). Open diamonds: Calculated OD values of the phbCAB mutant according to the difference in cell counts. 100 ml FSM medium in sealed glass bottles (pre-flushed with 2% oxygen and 98% nitrogen as described (R. Uebe et al., J. Bacteriol. 192:4192-4204, 2010) were inoculated from over-night cultures to a calculated optical density of 0.01 and incubated at 30°C and 120 rpm agitation. 1 ml samples were withdrawn at the indicated time points and measured photometrically for optical density and magnetic response as described in the Experimental Procedures section.

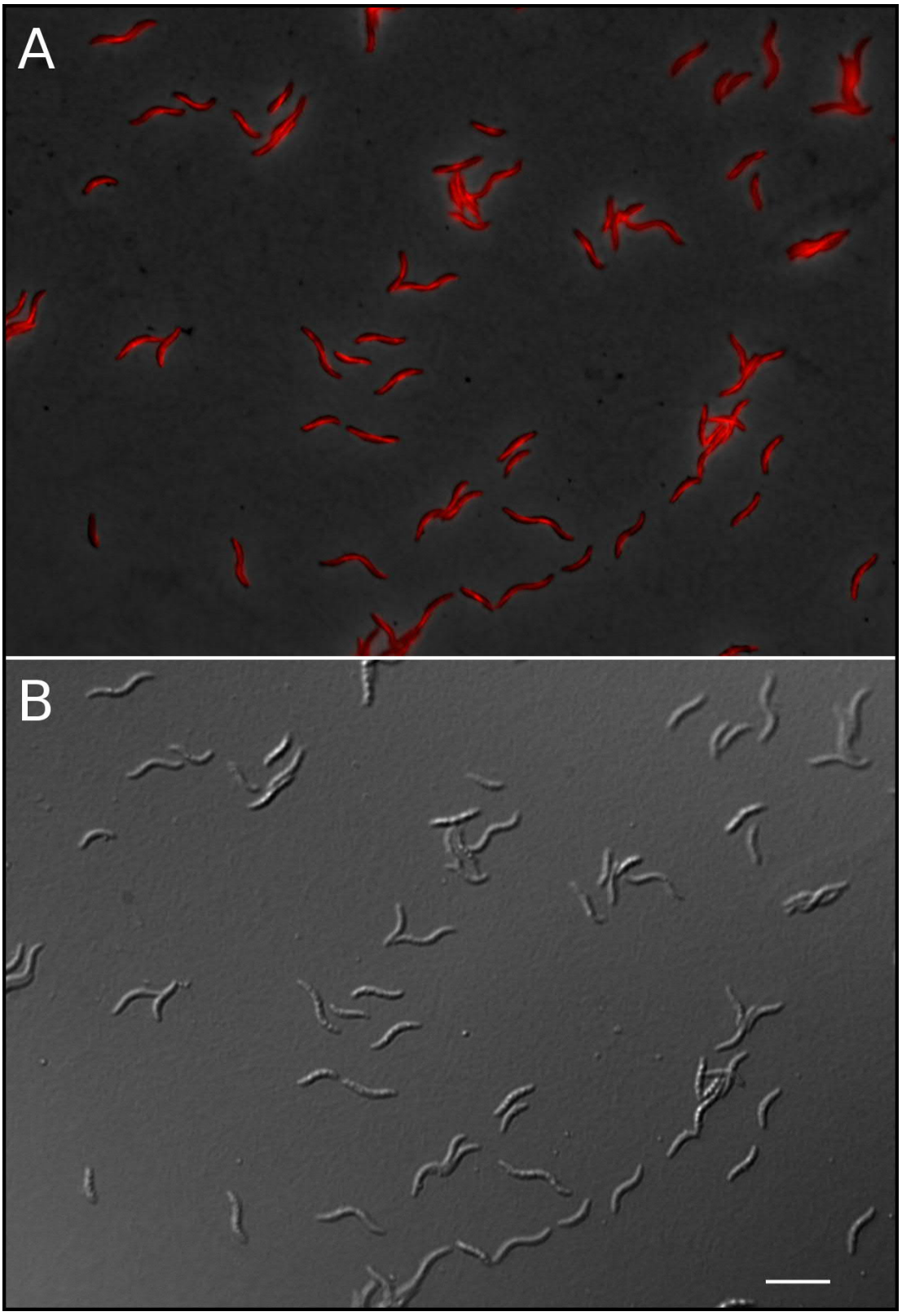

Figure S6. Chromosomal tagging of the actin-like mamK with mCherry results in a cell population with filamentous pole-to-pole fluorescence signals of uniform intensity and distribution. (A) Fluorescence and phase contrast overlay image of strain FM022 (mcherry-mamK). (B) DIC image of the same section. Bar: 5 µm.

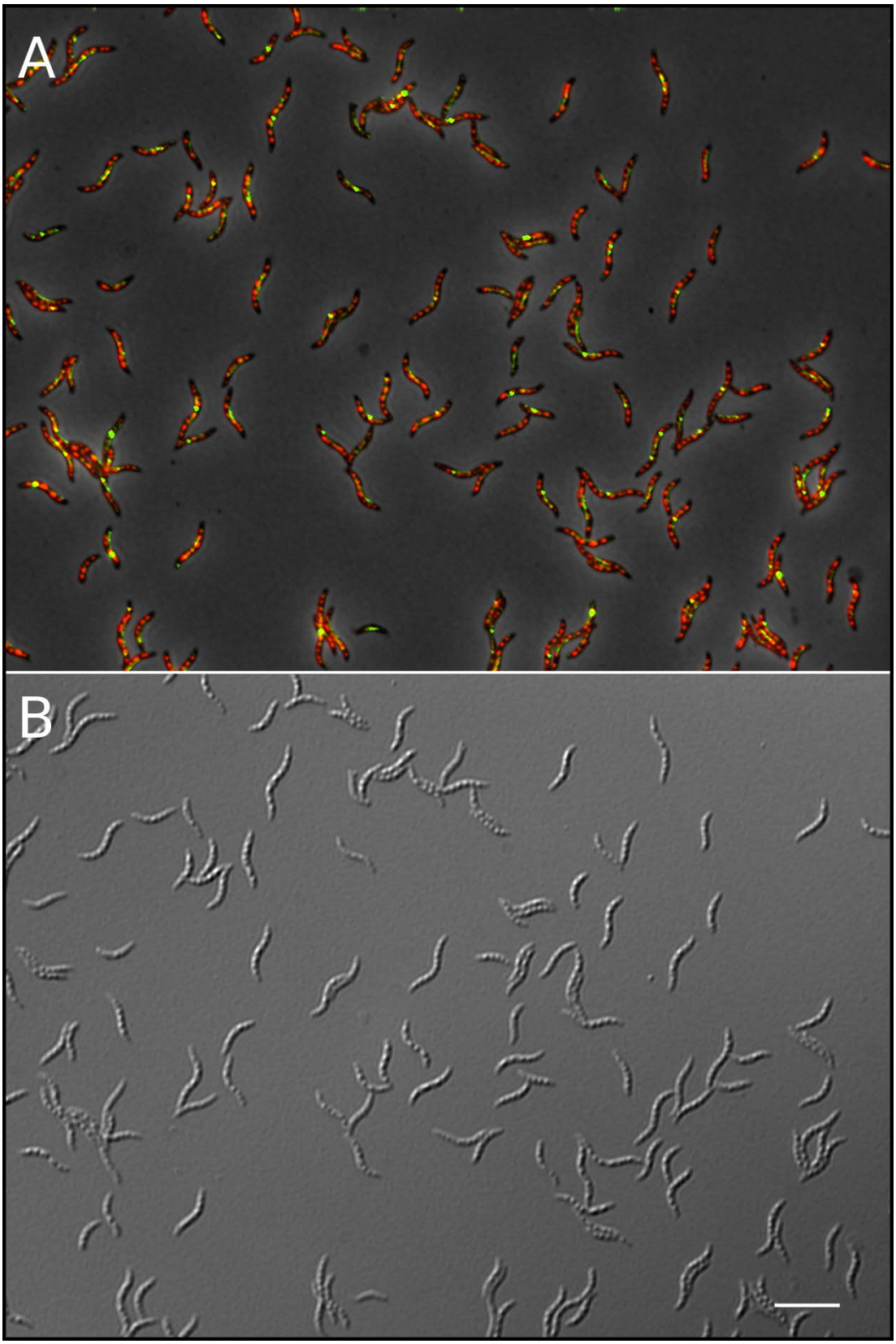
A
B

Figure S7. (A) Fluorescence and phase contrast overlay image of strain FM021 harbouring a chromosomally encoded fusion of the magnetosome membrane-specific MamC protein to EGFP. PHA inclusions were stained with Nile red. (B) DIC image of the same section. Bar: 5 µm.

85 **Table S1: DNA oligonucleotides**

Name	Sequence (5' → 3')[1]	Description
Primers for pORFM GalK / blu construction		
oOR059	gatgaagcttggcggatttgtcctactcagg	terminator amplification forward
oOR060	gactctgcaggactcctgttgatagatccagtaatgac	terminator amplification reverse
oOR063	cgagcatatgagtctgaaagaaaaaacacaatctc	*galK* forward
oOR077	acttggatccgttcagcactgtcctgctcc	*galK* reverse
oOR082	atgagtcgacaatttttgttgacactctatcattgatag	P_{tet}-*galK* forward
oOR083	gttccaattgcgttcagcactgtcctgctcc	P_{tet}-*galK* reverse
tetRfwSacI	gcagagctccttccggctggctggtttattg	*tetR* forward
tetRrevSacI	gctgagctcctttaagacccactttcac	*tetR* reverse
General pORFM screening and sequencing primers		
oFM280a	ctgccactcatcgcagtctagcttgg	pORFM GalK sequencing forward
oFM281	ggctttctacgtgttccgcttcctttagc	pORFM GalK sequencing reverse
oFM280b	aaacagctatgaccatgattacgccaagcg	pORFM blu sequencing forward
oFM281b	cgcgtaatacgactcactatagggcg	pORFM blu sequencing reverse
phbCAB deletion with pORFM blu		
oFM341	ggccggcgcgatcctcgacc	upstream fragment forward
oFM342	*acggcccac*atggcggtaaagggcgacgccg	upstream fragment reverse
oFM343	*taccgccat*gtgggccgtctgggccgcg	downstream fragment forward
oFM344	tggggcgggccacgtgctgc	downstream fragment reverse
oFM207g	gcccagggaatcaccgtaaaagccg	sequencing (upstream fragment)
oFM207h	gtcccggatgccccatcggc	sequencing (upstream fragment)

oFM207i	tcgggcgcggtattcagccgg	sequencing (downstream fragment)
oFM207k	ccatggcccaggccaatgccg	sequencing (downstream fragment)
oFM207l	ctgggtgaagatcttggcgaggaaattgg	verify deletion (upstream)
oFM207m	gccaggatcaaggcttgagtacccg	verify deletion (downstream)
mamC-egfp and *mamC-mCherry* in-frame fusion with pORFM GalK		
oFM270	gtcgacctagctatctgggcatcctctgcttcg	upstream fragment forward
oFM271	ggtaccggccaattcttccctcagaatgtcttcgtcg	upstream fragment reverse
oFM272	ggtaccgaacgttacgcgtcaccggtcggccacctgtg cctgcagggcgagctcgaggtgagcaagggcgagg agctgttc	*egfp* forward
oFM273	gaattcttatcacttgtacagctcgtccatgccgagag	*egfp* reverse
oFM274	gaattcaatattgggctggttcacggcattcagacacc	downstream fragment forward
oFM275	gctagccgacgaaggtggtcattttccaatgaccg	downstream fragment reverse
oFM276	ggtaccgaacgttacgcgtcaccggtcggccacctgtg cctgcagggcgagctcgaggtgagcaagggcgagg aggataacatgg	*mCherry* forward
oFM277	gaattctcattacttgtacagctcgtccatgccgcc	*mCherry* reverse
oFM278	atcggcggcatcggaaactggattgc	upstream fragment sequencing forward
oFM278a	tttcgtctcaggaaaggccaataccatgc	upstream fragment sequencing forward
oFM279	aatgacctcaggggaatcctctaccg	downstream fragment sequencing reverse
oFM279a	ggccttgggccttgtagatgtacg	downstream fragment sequencing reverse
oFM289a	gagcctgcttaagcgagggcaaagc	verify fusion (upstream)
oFM290	cgccattcatcgccttgtcgatgacg	verify fusion (downstream)
mCherry-mamK in-frame fusion with pORFM GalK		
oFM369	gtcgacggggcttcaggccaatgatcttatcatcg	*mamK-mCherry* fusion upstream fragment forward
oFM370	*cttgctcat*tttgtcactccgttcgcgtgctaacagatc	*mamK-mCherry* fusion

		upstream fragment reverse
oFM371	*agtgacaaa*atgagcaagggcgaggaggataacatgg	*mCherry* forward
oFM372	ggatccgcggccgccgaattctccggagctcgagatcttaaggtacccttgtacagctcgtccatgccgcc	*mCherry* reverse
oFM373	ggatcctatgagtgaaggtgaaggccaggccaag	*mCherry-mamK* fusion downstream fragment forward
oFM374	actagtgaacgccttcatcaccatgagcacgg	*mCherry-mamK* fusion downstream fragment reverse
oFM375	atgtccagccggaaacggatgccg	upstream fragment sequencing
oFM376	ggctatggcttggacaagctgaacaatacc	downstream fragment sequencing
oFM377	ctggggatggagatcgagacttttatggc	verify fusion (upstream)
oFM378	cccaataatcaccatcagggcgttcagc	verify fusion (downstream)

[1]Reverse complementary sequences are *italicised*, restriction sites are underlined.