SUPPORTING INFORMATION

A Screen for Protein-Protein Interactions in Live Mycobacteria Reveals a Functional Link Between the Virulence-Associated Lipid Transporter LprG and the Mycolyltransferase Antigen 85A

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SI Materials and Methods

GFP fluorescence measurements. A 10-mL culture of *Msm* transformed with pSMT-pHsp60-MjtRNA-3xFLAG and pMV361-pHsp60-MjTyrRS was grown in 7H9 for 9 hours at 37 °C at 225 rpm to an OD_{600} ~0.2. Filter-sterilized 100 mM pBpa in 1:5 1 N NaOH:water was added to the cells to a final concentration of 2 mM pBpa and incubated at 37 °C with shaking at 225 rpm in the dark for another 15 hours. Control cells were treated with an equivalent volume of 1:5 1 N NaOH:MQ water. Cells were harvested at 4,000 rpm and resuspended in PBS with 0.05% Tween80 to OD_{600} 1.0 and 150 µL were aliquoted in a 96-well plate (Corning). Fluorescence was measured using a Synergy 2 plate reader (BioTek) with an emission filter at 485 nm and an excitation filter at 538 nm.

Cloning. All LprG-6xHis constructs were generated by InFusion (Clontech) between vector digested with the specified enzymes and a gel-purified PCR product generated by the primers in Table S5. *Msm lprG* was first cloned into the *E. coli* vector pET24b (Novagen) to append a 6xHis tag and simultaneously insert a TEV protease cleavage site (ENLYFQS). The *lprG*-6xHis fusions were then subcloned into the mycobacterial shuttle plasmid pMV261 to generate the final expression constructs.

The pSMT-MsmLprG-3xFLAG-MjtRNA plasmid and its variants were developed from pSMT-MjtRNA-GFP(151TAG) (gift of Peter Schultz, Scripps Research Institute, La Jolla, California). DNA sequencing revealed that the TAG codon was located at amino acid 47, not 151; the plasmid was accordingly renamed pSMT-MjtRNA-GFP(47TAG). The *gfp* sequence was excised using restriction enzymes NdeI and XbaI, the 3xFLAG tag was amplified from pET24b pMop-3xFLAG using primers listed on Table S1 and subcloned into the pSMT vector via In-Fusion (Clontech) to create pSMT-3xFLAG-MjtRNA. The NdeI site was not reconstituted in the vector in order to maintain the unique nature of a second NdeI site in the insert. The gene for *lprG* (MSMEG_3070) was amplified from *Msm* mc^2155 genomic DNA using the primers listed on

Table S1 and subcloned into pSMT-3xFLAG-MjtRNA via In-Fusion (Clontech). The pMV361_MjTyrRS plasmid was constructed largely as described. MjTyrRS was amplified from the pEVOL-pBpF plasmid (Addgene, cat# 31190) and subcloned into pMV361 via In-Fusion (Clontech) using the primers listed on Table S1.

The pBpa and YH mutants were created by site-directed mutagenesis of *lprG* in pET24b-MsmLprG-TEV-6xHis or pMV261-MsmLprG-TEV-6xHis using the primers listed on Table S2. Mutated *lprG* was amplified and subcloned into pSMT-3xFLAG-MjtRNA using either In-Fusion or traditional ligation.

The constructs used to confirm crosslinking of LprG(98pBpa) with MS-identified interacting proteins were generated by amplifying the target gene from *Msm* mc^2155 genomic DNA using the primers listed in Table S3. The PCR products were subcloned into pSMT-MsmLprG(98pBpa)-3xFLAG-MjtRNA-6xHis by In-Fusion. For the additional LprG(pBpa) mutants LprG(136pBpa) and LprG(232pBpa), the corresponding alleles were excised from the appropriate pSMT-MsmLprG-3xFLAG-MjtRNA vector using NdeI and NheI. The pSMT-MsmLprG-Q98pBpa-3xFLAG-MjtRNA-(interactor)-6xHis (where “interactor” is *lprG*, *lppI*, or *lppk*) constructs were also digested with NdeI and NheI to remove *lprG*-Q98pBpa, and the *lprG*-A136pBpa or *lprG*-T232pBpa inserts were subcloned into this vector via traditional ligation to provide site-specific interaction controls. Sequence confirmed plasmids were electroporated into *Msm*::pMV361_MjTyrRS.

The *Msm* Δ*lprG* strain was generated as reported (*1*). Briefly, regions from the 5’ and 3’ ends of *lprG* were amplified from *Msm* mc^2155 genomic DNA using the primers listed in Table S3 and inserted on either side of the hygromycin resistance cassette in pJSC407 (*2*) by In-Fusion. The recombineering substrate was amplified using the 5’ flank forward primer and the 3’ flank reverse primer and 1 μg of the gel-purified product was transformed into *Msm.* Clones were recovered on selective medium and verified for recombination by PCR. The complement plasmid pMV306-*lprG* was generated using the primers in Table S3. Briefly, *lprG* and a 1kb

segment of the region upstream of the start codon (as a native promoter) was amplified and inserted into pMV306 (*3*) by In-Fusion. The sequence-verified plasmid was transformed into Δ*lprG* to generate the strain *ΔlprG::lprG* containing a single chromosomally integrated copy of *lprG* under control of a native promoter.

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SI Results

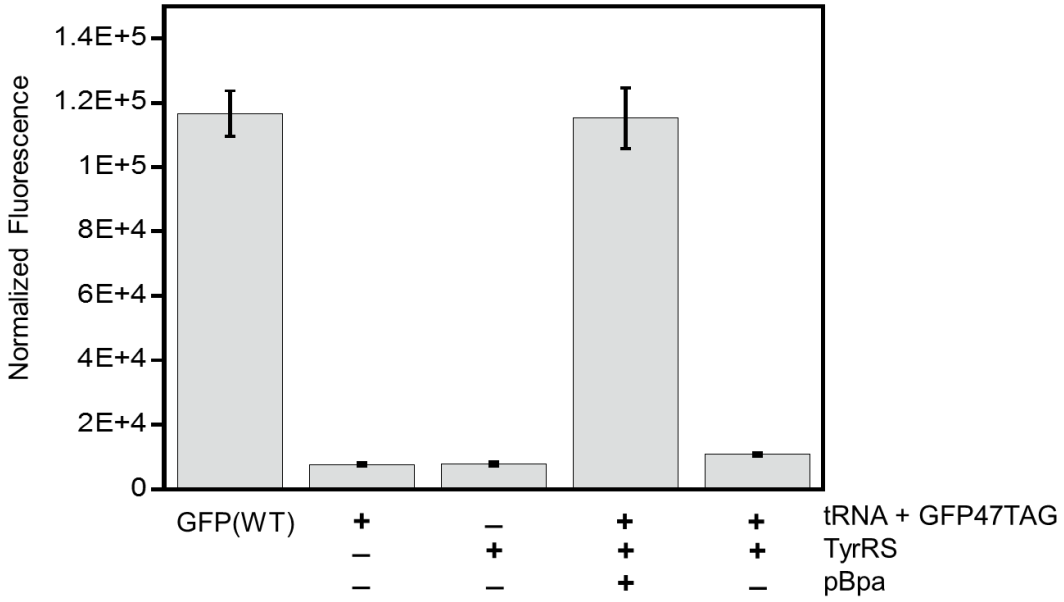

Figure S1. Validation of site-specific incorporation of the photocrosslinking unnatural amino acid pBpa into GFP47TAG via nonsense suppression in *Msm.* *Msm* expressing different components of the nonsense suppression machinery and either GFP(WT) or the mutant GFP47TAG were grown with or without 2 mM pBpa for 15 h. GFP fluorescence was measured in whole cells as a readout for expression of full-length GFP.

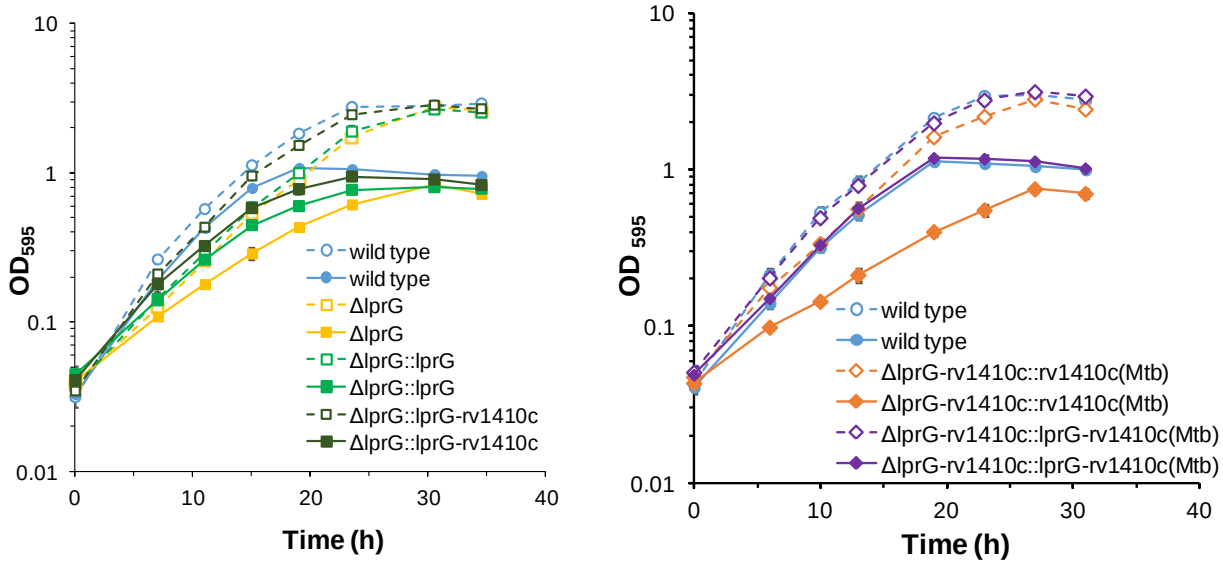

Figure S2. The growth attenuation of Δ*lprG* is complemented. (*left*) The introduction of a single copy of *lprG* in the Δ*lprG* deletion mutant partially restores growth on modified Sauton with either glycerol (dashed lines) or propionate (solid lines) as the primary carbon source. Complementation with both *lprG* and the downstream gene *rv1410c* further restores growth, suggesting that deletion of *lprG* compromises the expression of *rv1410c*, as has been noted previously (*4, 5*) . (*right*) In contrast, *Msm ΔlprG-rv1410c* (*6*) complemented with *Mtb rv1410c* shows a similar growth defect that is restored upon the addition of *Mtb lprG* (*7*). These additional data show that the observed growth attenuation is not due to loss of function in the downstream gene *rv1410c*.

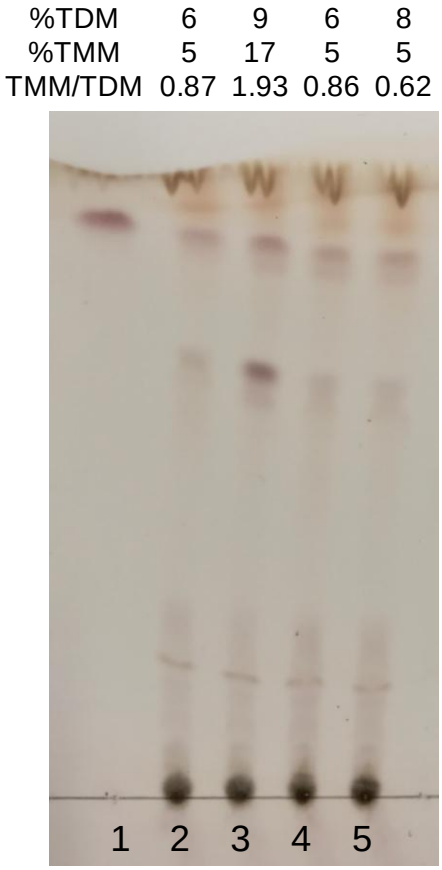

Figure S3. *ag85A::*Tn accumulates TMM without loss of TDM independent of the growth medium. TDM/TMM analysis was performed as in Figure 7C except the growth medium was 7H9 (see Methods). The calculated TDM and TMM levels (as a percent of total lipid) differed between experiments due to experimental variation in TLC resolution (especially for TDM). However, the relative lipid levels between strains were consistent, *i.e.*, with an elevated TMM level and TMM/TDM ratio for *ag85A*::Tn.

Table S1. LprG-6xHis expression constructs and oligonucleotide primers.

Vector_Construct_(Gene)	Forward primer (5' to 3')	Reverse primer (5' to 3')	Template
pET24b_MsmLprG-TEV-6xHis_(MSMEG_3070)	AAGAAGGAGATATACATATGCAGACGCGCCCACGC	GTGCGGCCGC***AAGCTT***GGATTGGAAGTACAGGTTTTCGGCCGCGGGCTTGG	*Msm* gDNA
pET24b_NA-MsmLprGTEV-6xHis_(MSMEG_3070)	AAGAAGGAGATATACATATGTCGTCGTCATCGGAGACCTCC	GTGCGGCCGC***AAGCTT***GGATTGGAAGTACAGGTTTTCGGCCGCGGGCTTGG	*Msm* gDNA
pMV261_MsmLprG-TEV-6xHis_(MSMEG_3070)	GGAATCACTTCGCAA**TGGCCA**GCCAGACGCGCCCACGC	ACATCGATAAGC**TTCGAA**TTCCTTTGTTAGCAGCCGGATCTCAGTG	pET24b_MsmLprG-TEV-6xHis_(MSMEG_3070)
pMV261_NA-MsmLprGTEV-6xHis_(MSMEG_3070)	GGAATCACTTCGCAA**TGGCCA**GCTCGTCGTCATCGGAGACCTCC	ACATCGATAAGC**TTCGAA**TTCCTTTGTTAGCAGCCGGATCTCAGTG	pET24b_NA-MsmLprGTEV-6xHis_(MSMEG_3070)

* Restriction sites: NdeI, ***HindIII***, **MscI**, **EcoRI**. In 3' primer: TEV cleavage site

Table S2. Unnatural amino acid incorporation expression constructs and oligonucleotide primers.

Mutation	Description	Forward primer (5' to 3')	Reverse primer (5' to 3')	Template
Y132pBpa	pSMT-pHsp60-MsmLprG-Y132pBpa-3xFLAG-MjtRNA	ggccaacatctaggacgtgtcggcgatcctg	ccgacacgtcctagatgttggccgcggcacc	pET24b-MsmLprG-TEV-6xHis
L70 pBpa	pSMT-pHsp60-MsmLprG-L70pBpa-3xFLAG-MjtRNA	ggtcgacgggtagcccgtggagaagctcgac	ctccacgggctacccgtcgaccttgccctg	pET24b-MsmLprG-TEV-6xHis
F96pBpa	pSMT-pHsp60-MsmLprG-F96pBpa-3xFLAG-MjtRNA	cctgatcgcgtagggccagaagatcgccgac	cttctggccctacgcgatcaggtcggcggtg	pMV261-pHsp60-MsmLprG-TEV-6xHis-MscI+EcoRI
Q98pBpa	pSMT-pHsp60-MsmLprG-Q98pBpa-3xFLAG-MjtRNA	cgcgttcggctagaagatcgccgacgcgaag	cggcgatcttctagccgaacgcgatcaggtc	pMV261-pHsp60-MsmLprG-TEV-6xHis-MscI+EcoRI
D102pBpa	pSMT-pHsp60-MsmLprG-D102pBpa-3xFLAG-MjtRNA	gaagatcgcctaggcgaagttcgtgatcgccg	cgaacttcgcctaggcgatcttctggccgaac	pMV261-pHsp60-MsmLprG-TEV-6xHis-MscI+EcoRI
L37pBpa	pSMT-pHsp60-MsmLprG-L37pBpa-3xFLAG-MjtRNA	ccgacgcaccgtagcccgacggtgccgcgctg	caccgtcgggctacggtgcgtcggaggtctccg	pET24b-MsmLprG-TEV-6xHis
N130pBpa	pSMT-pHsp60-MsmLprG-N130pBpa-3xFLAG-MjtRNA	gtgccgcggcctagatctacgacgtgtcggcg	cgtcgtagatctaggccgcggcaccgtagttc	pMV261-pHsp60-MsmLprG-TEV-6xHis-MscI+EcoRI
K187pBpa	pSMT-pHsp60-MsmLprG-K187pBpa-3xFLAG-MjtRNA	caccggcgctctaggccgacggcccggtgccc	ggccgtcggcctagagcgccggtgcgatcttg	pET24b-MsmLprG-TEV-6xHis
K181pBpa	pSMT-pHsp60-MsmLprG-K181pBpa-3xFLAG-MjtRNA	cgcggtcaactagatcgcaccggcgctcaag	ccggtgcgatctagttgaccgcgtcggcgctg	pMV261-pHsp60-MsmLprG-TEV-6xHis-MscI+EcoRI
A149pBpa	pSMT-pHsp60-MsmLprG-A149pBpa-3xFLAG-MjtRNA	caacgtgctgtagaacttctccgacgccacg	cggagaagttctacagcacgttggccaggc	pET24b-MsmLprG-TEV-6xHis

A136pBpa	pSMT-pHsp60-MsmLprG-A136pBpa-3xFLAG-MjtRNA	cgacgtgtcgtagatcctgaaccccgacacg	ggttcaggatctacgacacgtcgtagatgttg	pMV261-pHsp60-MsmLprG-TEV-6xHis-MscI+EcoRI
E160pBpa	pSMT-pHsp60-MsmLprG-E160pBpa-3xFLAG-MjtRNA	cgacggccgctagtcgatcaacggcaccgag	cgttgatcgactagcggccgtcggccgtggc	pET24b-MsmLprG-TEV-6xHis
D109pBpa	pSMT-pHsp60-MsmLprG-D109pBpa-3xFLAG-MjtRNA	cgtgatcgcctagggcaatctctacgcggc	gagattgccctaggcgatcacgaacttcgc	pMV261-pHsp60-MsmLprG-TEV-6xHis-MscI+EcoRI
L60pBpa	pSMT-pHsp60-MsmLprG-L60pBpa-3xFLAG-MjtRNA	cgtgcacctgtagctgacggtgcagggcaag	gcaccgtcagctacaggtgcacgctctgctgcg	pET24b-MsmLprG-TEV-6xHis
L93pBpa	pSMT-pHsp60-MsmLprG-L93pBpa-3xFLAG-MjtRNA	caccgccgactagatcgcgttcggccagaag	cgaacgcgatctagtcggcggtgccctccgc	pET24b-MsmLprG-TEV-6xHis
L75pBpa*	pSMT-pHsp60-MsmLprG-L75pBpa-3xFLAG-MjtRNA	cgtggagaagtaggacggcgacctgaccaac	ggtcgccgtcctacttctccacgggcagcccg	pET24b-MsmLprG-TEV-6xHis
V218pBpa	pSMT-pHsp60-MsmLprG-V218pBpa-3xFLAG-MjtRNA	gggcaacagctagacgatgacgctctcggac	gcgtcatcgtctagctgttgcccggcgtgggc	pET24b-MsmLprG-TEV-6xHis
K227pBpa	pSMT-pHsp60-MsmLprG-K227pBpa-3xFLAG-MjtRNA	gactggggttagcaggtcaacgtcaccaag	cgttgacctgctaaccccagtccgagagcg	pET24b-MsmLprG-TEV-6xHis
T232pBpa	pSMT-pHsp60-MsmLprG-T232pBpa-3xFLAG-MjtRNA	gaatcacttcgcacatatgcagacgcgcccac	ccttgtagtcgctagcggccgcgggcttc	pET24b-MsmLprG-TEV-6xHis
A236pBpa	pSMT-pHsp60-MsmLprG-A236pBpa-3xFLAG-MjtRNA	gaatcacttcgcacatatgcagacgcgcccac	ccttgtagtcgctagcctacgcgggcttggtg	pET24b-MsmLprG-TEV-6xHis
V23pBpa	pSMT-pHsp60-MsmLprG-V23pBpa-3xFLAG-MjtRNA	gcgctgtaggccgggtgttcgtcgtcatcggagacctccgacg	cccggcctacagcgcagcagcggtggcaaggatgg	pET24b-MsmLprG-TEV-6xHis
S29pBpa	pSMT-pHsp60-MsmLprG-S29pBpa-3xFLAG-MjtRNA	tcgtcgtagtcggagacctccgacgcaccgcttccgacg	ctccgactacgacgaacacccggcgaccagcgcagcagc	pET24b-MsmLprG-TEV-6xHis
E31pBpa	pSMT-pHsp60-MsmLprG-E31pBpa-3xFLAG-MjtRNA	tcatcgtagacctccgacgcaccgcttcccgacggtgccg	ggaggtctacgatgacgacgaacacccggcgaccagcgcagc	pET24b-MsmLprG-TEV-6xHis
T32pBpa	pSMT-pHsp60-MsmLprG-T32pBpa-3xFLAG-MjtRNA	catcggagtagtccgacgcaccgcttcccgacggtgcc	gtcggactactccgatgacgacgaacacccggcgaccagcg	pET24b-MsmLprG-TEV-6xHis
D34pBpa	pSMT-pHsp60-MsmLprG-D34pBpa-3xFLAG-MjtRNA	acctcctaggcaccgcttcccgacggtgccg	cggtgcctaggaggtctccgatgacgacgaacacccggc	pET24b-MsmLprG-TEV-6xHis
Y115H	pSMT-pHsp60-MsmLprG-Y115H-3xFLAG-MjtRNA	cggcaatctccacgcggcgctgacgcccggcg	cagcgccgcgtggagattgccgtcggcgatcac	pET24b-MsmLprG-TEV-6xHis
Y125H	pSMT-pHsp60-MsmLprG-Y127H-3xFLAG-MjtRNA	gctgtcgaaccacggtgccgcggccaacatc	gccgcggcaccgtggttcgacagcggatcgccg	pET24b-MsmLprG-TEV-6xHis

Y132H	pSMT-pHsp60-MsmLprG-Y132H-3xFLAG-MjtRNA	gccaacatccacgacgtgtcggcgatcctg	gacacgtcgtggatgttggccgcggcacc	pET24b-MsmLprG-TEV-6xHis
Y2xH (Y115H + Y125H)	pSMT-pHsp60-MsmLprG-Y115H-Y127H-3xFLAG-MjtRNA	gctgtcgaaccacggtgccgcggccaacatc	gccgcggcaccgtggttcgacagcggatcgccg	pSMT-pHsp60-MsmLprG-Y115H-3xFLAG-MjtRNA
Y3xH (Y115H + Y125H + Y132H)	pSMT-pHsp60-MsmLprG-Y115H-Y125H-Y132H-3xFLAG-MjtRNA	gccaacatccacgacgtgtcggcgatcctg	gacacgtcgtggatgttggccgcggcacc	pSMT-pHsp60-MsmLprG-Y115H-Y125H-3xFLAG-MjtRNA

*Traditional ligation

Table S3. Unnatural amino acid incorporation expression constructs and oligonucleotide primers used to confirm MS-identified LprG-interactor proteins.

Interactor	Description	Forward primer (5' to 3')	Reverse primer (5' to 3')
LprG	pSMT-pHsp60-MsmLprG-Q98pBpa-3xFLAG-hsp60-lprG-6xHis-MjtRNA	tcacttcgcaacgcgtgcagacgcgcccac	ggtgatggtgggatccggccgcgggcttggtg
LppI	pSMT-pHsp60-MsmLprG-Q98pBpa-3xFLAG-hsp60-lppI-6xHis-MjtRNA	tcacttcgcaacgcgtgcggactgctgtgatcctc	ggtgatggtgggatccatccggttcgcagctgaac
LppK	pSMT-pHsp60-MsmLprG-Q98pBpa-3xFLAG-hsp60-lppK-6xHis-MjtRNA	tcacttcgcaacgcgtgaaccggaaccgaatcgcag	ggtgatggtgggatccgactgcccgccaccgg
Ag85A	pSMT-Hsp60-LprG- Q98ΔTAG-Flag, Hsp60-ag85A-6xHis-MjtRNA	tcacttcgcaacgcgtgaagttcgttgggagaatgcgcg	tggtgatggtgggatccggcggtcggggtcgc

Table S4. Constructs and oligonucleotide primers used for the production of the Δ*lprG* and Δ*lprG*:*lprG*

Construct	Description	Note	Forward primer (5' to 3')	Reverse primer (5' to 3')	Template
Δ*lprG* recombineering substrate	pJSC407-Δ*lprG*	5' flank inserted using HindIII	tggatccacgaagcttggatggtcagacgggcgg	ggccaccatgaagcttctaggtggcaaggatggcgaac	*Msm* mc^{2}155 genomic DNA
		3' flank inserted using XbaI	cggacaggactctagagactggggtaagcaggtcaac	ccggggatcctctagaggtttatcgcgatgccgacg	*Msm* mc^{2}155 genomic DNA
***lprG* complement plasmid**	pMV306-*lprG*	inserted using XbaI and **ClaI**	gatctttaaatctagaccagccggtcgcagatc	actacgtcgac**atcgat**tcaggccgcgggc	*Msm* mc^{2}155 genomic DNA