

***E. coli:***

Strain	Description	Reference
DH5 $\alpha$	F <sup>-</sup> , p80 <i>lacZ</i> ΔM15 Δ( <i>lacZYA-argF</i> )U169 <i>deoR recA1 endA1 hsdR17</i> ( <i>r<sub>k</sub>-m<sub>k</sub>+ </i> ) <i>phoA supE44 λ- thi-1 gyrA96 relA1</i>	GIBCO, BRL
ER2566	F- <i>λ- fhuA2</i> [lon] <i>ompT lacZ::T7 gene1 gal sulA11</i> Δ( <i>mcrC-mrr</i> )114::IS10 R( <i>mcr-73::miniTn10</i> )2 R( <i>zgb-210::Tn10</i> )(Tets) <i>endA1</i> [dcm]	(1)

***M. tuberculosis:***

Strain	Description	Reference
H37Rv	WT ATCC collection #25618	ATCC
MHD2	Kan <sup>R</sup> ; <i>pafA::ΦMycoMarT7</i>	(2)
MHD5	Kan <sup>R</sup> ; <i>mpa::ΦMycoMarT7</i>	(2)
MHD802	Hyg <sup>R</sup> ; Δ <i>prcBA::hyg</i>	(3)
MHD827	Hyg <sup>R</sup> ; H37Rv, pOLYG-WT ΔPP <i>prcB</i> (FLAG-GGGG-His <sub>6</sub> )A	This study
MHD828	Hyg <sup>R</sup> ; H37Rv, pOLYG-T1A ΔPP <i>prcB</i> (FLAG-GGGG-His <sub>6</sub> )A	This study
MHD829	Kan <sup>R</sup> , Hyg <sup>R</sup> ; MHD2, pOLYG-WT ΔPP <i>prcB</i> (FLAG-GGGG-His <sub>6</sub> )A	This study
MHD830	Kan <sup>R</sup> , Hyg <sup>R</sup> ; MHD2, pOLYG-T1A ΔPP <i>prcB</i> (FLAG-GGGG-His <sub>6</sub> )A	This study
MHD831	Kan <sup>R</sup> , Hyg <sup>R</sup> ; MHD5, pOLYG-WT ΔPP <i>prcB</i> (FLAG-GGGG-His <sub>6</sub> )A	This study
MHD832	Kan <sup>R</sup> , Hyg <sup>R</sup> ; MHD5, pOLYG-T1A ΔPP <i>prcB</i> (FLAG-GGGG-His <sub>6</sub> )A	This study
MHD872	Hyg <sup>R</sup> ; H37Rv, pOLYG-His <sub>6</sub> - <i>pafE</i>	This study
MHD879	Hyg <sup>R</sup> ; H37Rv, pOLYG-His <sub>6</sub> - <i>pafE</i> <sub>Y173A</sub>	This study
MHD920	Kan <sup>R</sup> ; H37Rv, L5 <i>attB::Rv3781-Rv3783</i>	This study
MHD924	Kan <sup>R</sup> , Hyg <sup>R</sup> ; MHD5, L5 <i>attB::mpa</i> <sub>L604A</sub>	This study
MHD925	Kan <sup>R</sup> , Hyg <sup>R</sup> ; MHD5, L5 <i>attB::mpa</i> <sub>Q605A</sub>	This study
MHD926	Kan <sup>R</sup> , Hyg <sup>R</sup> ; MHD5, L5 <i>attB::mpa</i> <sub>Q606A</sub>	This study
MHD927	Kan <sup>R</sup> , Hyg <sup>R</sup> ; MHD5, L5 <i>attB::mpa</i> <sub>Y607A</sub>	This study
MHD928	Kan <sup>R</sup> , Hyg <sup>R</sup> ; MHD5, L5 <i>attB::mpa</i> <sub>L608A</sub>	This study
MHD929	Kan <sup>R</sup> , Hyg <sup>R</sup> ; MHD5, L5 <i>attB::mpa</i> <sub>ΔL608</sub>	This study
MHD930	Kan <sup>R</sup> , Hyg <sup>R</sup> ; MHD5, L5 <i>attB::mpa</i> <sub>Q606L</sub>	This study
MHD931	Kan <sup>R</sup> , Hyg <sup>R</sup> ; MHD5, L5 <i>attB::mpa</i> <sub>K591R</sub>	This study
MHD932	Kan <sup>R</sup> , Hyg <sup>R</sup> ; MHD920, Δ <i>pafE::hyg</i>	This study
MHD946	Kan <sup>R</sup> , Strep <sup>R</sup> ; MHD920, pAJF381	This study
MHD947	Kan <sup>R</sup> , Hyg <sup>R</sup> , Strep <sup>R</sup> ; MHD932, pAJF381- <i>pafE</i>	This study
MHD980	Kan <sup>R</sup> , Hyg <sup>R</sup> , Strep <sup>R</sup> ; MHD932, pAJF381	This study
MHD981	Kan <sup>R</sup> , Hyg <sup>R</sup> , Strep <sup>R</sup> ; MHD932, pAJF381- <i>pafE</i> <sub>Y173A</sub>	This study

## Plasmids:

Plasmid	Description	Reference
pET-24b(+)	Kan <sup>R</sup> ; For inducible production of recombinant protein in <i>E. coli</i>	Novagen
pET-24b(+)-His <sub>6</sub> - <i>pafE</i>	Kan <sup>R</sup> ; For purification of recombinant PafE with an N-terminal, thrombin-cleavable His <sub>6</sub> -tag	This study
pET-24b(+)-His <sub>6</sub> - <i>pafE</i> <sub>G171A</sub>	Kan <sup>R</sup> ; For purification of recombinant, mutated PafE with an N-terminal, thrombin-cleavable His <sub>6</sub> -tag	This study
pET-24b(+)-His <sub>6</sub> - <i>pafE</i> <sub>Q172A</sub>	Kan <sup>R</sup> ; For purification of recombinant, mutated PafE with an N-terminal, thrombin-cleavable His <sub>6</sub> -tag	This study
pET-24b(+)-His <sub>6</sub> - <i>pafE</i> <sub>Y173A</sub>	Kan <sup>R</sup> ; For purification of recombinant, mutated PafE with an N-terminal, thrombin-cleavable His <sub>6</sub> -tag	This study
pET-24b(+)-His <sub>6</sub> - <i>pafE</i> <sub>L174A</sub>	Kan <sup>R</sup> ; For purification of recombinant, mutated PafE with an N-terminal, thrombin-cleavable His <sub>6</sub> -tag	This study
pET-24b(+)-His <sub>6</sub> - <i>pafE</i> <sub>ΔL174</sub>	Kan <sup>R</sup> ; For purification of recombinant, mutated PafE with an N-terminal, thrombin-cleavable His <sub>6</sub> -tag	This study
pET-32a(+)	Amp <sup>R</sup> ; For inducible production of recombinant protein in <i>E. coli</i>	Novagen
pET-32a(+)-WT ΔPP <i>prcB</i> (His <sub>6</sub> )A	Amp <sup>R</sup> ; For purification of recombinant, active, His <sub>6</sub> -tagged 20S	This study
pET-32a(+)-20S <sub>OG</sub> CP	Amp <sup>R</sup> ; For purification of recombinant, active, His <sub>6</sub> -tagged open-gate 20S	This study
pET-32a(+)-HspR-GGGG-His <sub>6</sub>	Amp <sup>R</sup> ; For purification of recombinant His <sub>6</sub> -tagged HspR	This study
pOLYG	Hyg <sup>R</sup> ; For overproduction of protein in <i>M. tuberculosis</i>	(4)
pOLYG-WT ΔPP <i>prcB</i> (FLAG-GGGG-His <sub>6</sub> )A	Hyg <sup>R</sup> ; For overproduction of active, TAP-tagged 20S in <i>M. tuberculosis</i> . Contains 200 bp of promoter region upstream of <i>pup</i>	This study
pOLYG-T1A ΔPP <i>prcB</i> (FLAG-GGGG-His <sub>6</sub> )A	Hyg <sup>R</sup> ; For overproduction of inactive, TAP-tagged 20S in <i>M. tuberculosis</i> . Contains 200 bp of promoter region upstream of <i>pup</i>	This study
pOLYG-His <sub>6</sub> - <i>pafE</i>	Hyg <sup>R</sup> ; For overproduction of His <sub>6</sub> -tagged PafE in <i>M. tuberculosis</i> . Contains 200 bp of promoter region upstream of <i>pafE</i>	This study
pOLYG-His <sub>6</sub> - <i>pafE</i> <sub>Y173A</sub>	Hyg <sup>R</sup> ; For overproduction of His <sub>6</sub> -tagged PafE <sub>Y173A</sub> in <i>M. tuberculosis</i> . Contains 200 bp of sequence upstream of <i>pafE</i>	This study
pMV306-kan	Kan <sup>R</sup> ; For integration into the L5 <i>attB</i> site of the <i>M. tuberculosis</i> chromosome	(5)
pMV306-kan-Rv3781-Rv3783	Kan <sup>R</sup> ; To introduce the Rv3781-Rv3783 region into the chromosomal L5 <i>attB</i> site. Contains 200 bp of the putative promoter region upstream of Rv3779	This study
pMV306	Hyg <sup>R</sup> ; For integration into the L5 <i>attB</i> site of the <i>M. tuberculosis</i> chromosome	(5)
pMV306- <i>mpa</i> <sub>L604A</sub>	Hyg <sup>R</sup> ; To complement MHD5 by introducing mutated <i>mpa</i> variants into the chromosomal L5 <i>attB</i> site. Contains 200 bp of the region upstream of <i>mpa</i> .	This study
pMV306- <i>mpa</i> <sub>G605A</sub>	Hyg <sup>R</sup> ; To complement MHD5 by introducing mutated <i>mpa</i> variants into the chromosomal L5 <i>attB</i> site. Contains 200 bp of the region upstream of <i>mpa</i> .	This study
pMV306- <i>mpa</i> <sub>Q606A</sub>	Hyg <sup>R</sup> ; To complement MHD5 by introducing mutated <i>mpa</i> variants into the chromosomal L5 <i>attB</i> site. Contains 200 bp of the region upstream of <i>mpa</i> .	This study
pMV306- <i>mpa</i> <sub>Y607A</sub>	Hyg <sup>R</sup> ; To complement MHD5 by introducing mutated <i>mpa</i> variants into the chromosomal L5 <i>attB</i> site. Contains 200 bp of the region upstream of <i>mpa</i> .	This study
pMV306- <i>mpa</i> <sub>L608A</sub>	Hyg <sup>R</sup> ; To complement MHD5 by introducing mutated <i>mpa</i> variants into the chromosomal L5 <i>attB</i> site. Contains 200 bp of the region upstream of <i>mpa</i> .	This study
pMV306- <i>mpa</i> <sub>ΔL608</sub>	Hyg <sup>R</sup> ; To complement MHD5 by introducing mutated <i>mpa</i> variants into the chromosomal L5 <i>attB</i> site. Contains 200 bp of the region upstream of <i>mpa</i> .	This study
pMV306- <i>mpa</i> <sub>Q606L</sub>	Hyg <sup>R</sup> ; To complement MHD5 by introducing mutated <i>mpa</i> variants into the chromosomal L5 <i>attB</i> site. Contains 200 bp of the region upstream of <i>mpa</i> .	This study
pYUB854	Hyg <sup>R</sup> ; To generate an <i>M. tuberculosis</i> mutant through double-crossover recombination	(6)
pYUB854-Δ <i>pafE</i> :: <i>hyg</i>	Hyg <sup>R</sup> ; To delete and disrupt the <i>pafE</i> gene locus with a hygromycin resistance cassette. Leaves intact the first 30 and the last 65 base pairs of the coding sequence.	This study
pAJF381	Strep <sup>R</sup> ; For integration into the Tweety <i>attB</i> site of the <i>M. tuberculosis</i> chromosome	Allison Faye and Michael Glickman
pAJF381- <i>pafE</i>	Strep <sup>R</sup> ; To complement MHD932 by introducing <i>pafE</i> into the chromosomal Tweety <i>attB</i> site. Contains 200 bp of promoter region upstream of Rv3779.	This study
pAJF381- <i>pafE</i> <sub>Y173A</sub>	Strep <sup>R</sup> ; To complement MHD932 by introducing mutated <i>pafE</i> into the chromosomal Tweety <i>attB</i> site. Contains 200 bp of promoter region upstream of Rv3779.	This study

## Primers:

Primer Name	Primer sequence
<i>prcB</i> Promoter HindIII F	agAAGCTTgtagtaaggcgcatgtgg
<i>prcB</i> WT SOE R	tcagcgcgacaatggtggCcatatgtatatctccttcgccgcctcctgcttcc
<i>prcB</i> WT SOE F	ggaagcaggaggcggcgaaggagatatacatatgGccaccattgtcgcgctga
<i>prcB</i> T1A SOE R	tcagcgcgacaatggtggTcatatgtatatctccttcgccgcctcctgcttcc
<i>prcB</i> T1A SOE R	ggaagcaggaggcggcgaaggagatatacatatgAccaccattgtcgcgctga
<i>prcB</i> -TAP-NheI SOE R	atGCTAGCtcaATGATGATGATGATGATGATGTCCTCCTCCTCCcttgtcatcgtcatccttgaatccttctcacc
<i>prcB</i> -NheI- <i>prcA</i> SOE F	CATCATCATCATtgaGCTAGCgaaggagatatacatatgtagtittccgtatttc
<i>prcA</i> BamHI F	taGGATCCgaaggagatatacatatgtagtittccgtatttc
RBS- <i>prcA</i> OG KpnI F	ataGGTACCctttaagaaggagatatacaatggagcaggcgatgcgcgagcgcagcgagtgg
<i>prcA</i> XbaI R	actctagatcagccccgacgattcg
<i>mpa</i> promoter KpnI F	TAGGTACCccactacgcctgtgctagg
<i>mpa</i> HindIII R	TGAAGCTTtctacaggctggtggcggattg
thrHis- <i>pafE</i> NdeI F	TTACATATGCATCATCATCATCATCATCTAGTACCAAGAGGTAGTGTGATTGGATTGAGTACC
<i>pafE</i> HindIII R	TATAAGCTTTTACAGGTATTGTCCGGTGC
<i>pafE</i> promoter HindIII F	TTAAAGCTTGGCATCCTCGGGTACTCG
<i>pafE</i> BamHI R	TATGGATCCTTACAGGTATTGTCCGGTGC
<i>pafE</i> pYUB 5' StuI F	tctaggcctAGTTCCTTCTATCACGATCC
<i>pafE</i> pYUB 5' XbaI R	tagtctagaGACGTCGTGTCGTGTCGTGCCGGTACTC
<i>pafE</i> pYUB 3' HindIII F	tataagcttGTGCGCTGCCGCCCGGGGTGCGCAAGTC
<i>pafE</i> pYUB 3' XhoI R	tatctcgagGCATAGTCGAGCCAAAACTC
<i>pafE</i> Y173A HindIII R	TATAAGCTTTTACAGGGCTTGTCCGGTGC
<i>pafE</i> Y173A BamHI R	TATGGATCCTTACAGGGCTTGTCCGGTGC
<i>rv3779</i> promoter XbaI F	tagtctagaACACGGTGGTCGCGACGGAATCG
<i>rv3781</i> SOE R	GGATGGTGCGGATCCGACACTCGATTAGTATGGCTGGCCGGGTGACATGG
<i>rv3781</i> SOE F	CCATGTCACCCCGCCAGCCATACTAATCGAGTGTCCGATCCGCACCATCC
<i>rv3783</i> HindIII R	tagaagcttCTACACCCAGTAGGGCACCCGCGCGGATAC
<i>mpa</i> L604A HindIII R	atcaagcttCTACAGGTACTGGCCGGCGTTGG
<i>mpa</i> G605A HindIII R	atcaagcttCTACAGGTACTGGCCGAGGTTGG
<i>mpa</i> Q606A HindIII R	atcaagcttCTACAGGTACGCGCCGAGGTTGG
<i>mpa</i> Y607A HindIII R	atcaagcttCTACAGGGCCTGGCCGAGGTTGG
<i>mpa</i> L608A HindIII R	atcaagcttCTACGCGTACTGGCCGAGGTTGG
<i>mpa</i> ΔL608 HindIII R	atcaagcttCTAGTACTGGCCGAGGTTGG
<i>mpa</i> Q606L HindIII R	atcaagcttCTACAGGTACAGGCCGAGGTTGG
<i>mpa</i> K591R HindIII R	atcaagcttCTACAGGTACTGGCCGAGGTTGGACTCGGTATCAATAGCCCTGCTGGCCGACGAACT CCTTCC
<i>rv3779</i> prom SpeI F	AGAAGTAGTACACGGTGGTCGCGACGGAATCG
<i>pafE</i> SOE R	CCTTCTAGGAGTGTGTTGCTGCTCGATTAGTATGGCTGGCCGGGTGACATGG
<i>pafE</i> SOE F	CCATGTCACCCCGCCAGCCATACTAATCGAGCAGCAACACACTCCTAGAAGG
<i>pafE</i> KpnI R	TATGGTACCTTACAGGTATTGTCCGGTGC
<i>pafE</i> Y173A KpnI R	TATGGTACCTTACAGGGCTTGTCCGGTGC
<i>pafE</i> T170A HindIII R	TATAAGCTTTTACAGGTATTGTCCGGCGCCGTGGCCGTG
<i>pafE</i> G171A HindIII R	TATAAGCTTTTACAGGTATTGTCCGGTGGCCGTGGCCGTG
<i>pafE</i> Q172A HindIII R	TATAAGCTTTTACAGGTATGCTCCGGTGGCCGTGGCCGTG
<i>pafE</i> L174A HindIII R	TATAAGCTTTTACGCGTATTGTCCGGTGGCCGTGGCCGTG
<i>pafE</i> ΔL174 HindIII R	TATAAGCTTTTGTATTGTCCGGTGGCCGTGGCCGTG