Convergence of two global regulators to coordinate expression of essential virulence determinants of *Mycobacterium tuberculosis*

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Running title: Regulation of mycobacterial virulence determinants

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Supplementary file 1a

Oligonucleotide primers used for amplification and cloning in this study

|  |  |  |
| --- | --- | --- |
| **Primersa** | **Sequences (5’-3’)** | **Reference** |
| FPwhiB1up | AATAATAAGCTTAGATGTGATGGG | This study |
| RPwhiB1up | AATAATGGTACCTACCGGGAAGAA | This study |
| FPwhiB1upmut | GTCAAACAAGGATCACAAAACGAGATCGCCA | This study |
| RPwhiB1upmut | TTGTGATCCTTGTTTGACTGTACTACACTA | This study |
| FPwhiB1up1 | AATAATAAGCTTAGATGTGATGGG | This study |
| RPwhiB1up1 | AATAATGGTACCAGCGCGTGGGCT | This study |
| FPwhiB1up2 | AATAATGGATCCTACGTAACACTA | This study |
| RPwhiB1up2 | AATAATGGTACCAGCGCGTGGGCT | This study |
| FPwhiB1up3 | AATAATTCCGAAGAAACGCCT | This study |
| FPphoPstart | AATAATGGATCCATGCGGAAAGGGGT | This study |
| RPphoPFLAG | AATAAGCTTTCACTTGTCGTCATCGTCTTTGTAGTCTCGAGGCTCCCGCA | This study |
| FPmCMR | AATAATTGTTCGACTGACG | This study |
| RPmCMR | AATAATCGGGTTTGTGTTGT | This study |
| FPmCRP | AATAATAAGCTTGTGGACGAGATC | This study |
| RPmCRP | AATAATATCGATTTACCTCGCTCG | This study |
| FPPhoPN | CCTGGATCCATGCGGAAAGGGGTT | ([Pathak et al., 2010](#_ENREF_7)) |
| RPPhoPN | AATAATCTCGAGGCGTCGCAGGATGA | This study |
| FPPhoPC | AATAATCGGATCCAAGGGCAACAAGGAACCA | This study |
| RPPhoPC | GGTCTCGAGTCGAGGCTCCCGCAG | ([Pathak et al., 2010](#_ENREF_7)) |
| FPCRP | AATAATCATATGGTGGACGAGATC | This study |
| FPCRPN | ATATATGGATCCCCCGTCGACTTCCCCC | This study |
| RPCRPN | ATATATAAGCTTTCAACGATCGGCGAT | This study |
| FPCRPC | ATATATCATATGGTGCCCGGTCGGGT | This study |
| RPCRP | AATAATCTCGAGTTACCTCGCTCG | This study |
| FPCRPG79A | CATGTTCGCGGAGTTGTCGATCT | This study |
| RPCRPG79A | CGACAACTCCGCGAACATGTCCGA | This study |
| FPCRPT90A | GTCCGCGCGCGTCCAGC | This study |
| RPCRPT90A | GCTGGACGCGCGCGGACCCGGGT | This study |
| FPPhoP62-64A | GCAGCAGCACCGGACGCGGTG | This study |
| RPPhoP62-64A | TGCTGCTGCCCGGGCCCGATC | This study |
| FPPhoP76-78A | GCAGCAGCAGGCTTTGGGGTG | This study |
| RPPhoP76-78A | TGCTGCTGCGGGCATCATCAC | This study |
| FPPhoP105-107A | GCAGCAGCAATCGCGGGTCTG | This study |
| RPPhoP105-107A | TGCTGCTGCTAGCGAGTCACG | This study |
| FPPhoP110-112A | GCAGCAGCACTGGGTGGTGAC | This study |
| RPPhoP110-112A | TGCTGCTGCCGCGATCTTGTC | This study |
| FPPhoP118-120A | GCAGCAGCAAAGCCCTTCAGT | This study |
| RPPhoP118-120A | TGCTGCTGCGTCGTCACCACC | This study |

aFP, forward primer; RP, reverse primer

Supplementary file 1b

Plasmids used in this study

|  |  |  |
| --- | --- | --- |
| **Plasmids** |  |  |
| pET-*phoP*a | His6 tagged-PhoP residues 1-247 cloned in pET15b | ([Gupta et al., 2009](#_ENREF_6)) |
| pGEX-*phoP* | PhoP residues 1-247 cloned in pGEX-4T-1 | ([Gupta et al., 2009](#_ENREF_6)) |
| pGEX-*phoP*LAla5 | G142-P146 residues mutated to A in *phoP* of pGEX-*phoP* | This study |
| pGEX-*phoPN* | PhoP residues 1-141 cloned in pGEX-4T-1 | This study |
| pGEX-*phoPC* | PhoP residues 141-247 cloned in pGEX-4T-1 | This study |
| pGEX-*phoP*(62-64)Ala | E62-R64 residues mutated to A in *phoP* of pGEX-*phoP* | This study |
| pGEX-*phoP*(76-78)Ala | G76-D78 residues mutated to A in *phoP* of pGEX-*phoP* | This study |
| pGEX-*phoP*(105-107)Ala | Q105-K107 residues mutated to A in *phoP* of pGEX-*phoP* | This study |
| pGEX-*phoP*(110-112)Ala | G110-T112 residues mutated to A in *phoP* of pGEX-*phoP* | This study |
| pGEX-*phoP*(118-120)Ala | Y118-T120 residues mutated to A in *phoP* of pGEX-*phoP* | This study |
| pME1mL1-*phoP*b | PhoP residues 1-247 cloned in pME1mL1 | ([Goyal et al., 2011](#_ENREF_5)) |
| pSM128c | Integrative promoter probe vector for mycobacteria | ([Dussurget et al., 1999](#_ENREF_4)) |
| pSM-*whiB1*up | whiB1up-*lacZ* fusion in pSM128 | This study |
| pSM-*whiB1*upmut | pSM-*whiB1*up carrying changes in the PhoP binding site | This study |
| pUAB400d | Integrative mycobacteria - *E. coli* shuttle plasmid, Kanr | ([Singh et al., 2006](#_ENREF_8)) |
| pUAB400-*phoP* | PhoP residues 1-247 cloned in pUAB400 | ([Singh et al., 2014](#_ENREF_9)) |
| pUAB300b | Episomal mycobacteria - *E. coli* shuttle plasmid, Hygr | ([Singh et al., 2006](#_ENREF_8)) |
| pUAB300-*crp* | CRP residues 1-224 cloned in pUAB300 | This study |
| pUAB300-*cmr* | CMR residues 1-244 cloned in pUAB300 | This study |
| pET-28cd | *E. coli* cloning vector, Kanr | Novagen |
| pET-*crp*d | His6 tagged-CRP residues 1-224 cloned in pET-28c | This study |
| pET-*crpN* | His6 tagged -CRP residues 28-116 cloned in pET-28c | This study |
| pET-*crpC* | His6 tagged-CRP residues 146-224 cloned in pET-28c | This study |
| p19Kprob | Mycobacteria expression vector | ([De Smet et al., 1999](#_ENREF_3)) |
| p19Kpro-*phoP* | His6-tagged PhoP residues 1-247 cloned in p19Kpro | ([Anil Kumar et al., 2016](#_ENREF_1)) |
| p19Kpro-*phoP*FLAG | FLAG-tagged PhoP residues 1-247 cloned in p19Kpro | This study |

a ampicillin resistance; b hygromycin resistance

c streptomycin resistance; d kanamycin resistance

Supplementary file 1c

Sequence of oligonucleotide primers used in aRT-qPCR and bChIP-qPCR experiments reported in this study

|  |  |  |
| --- | --- | --- |
| Primersa | Sequences (5’-3’) | Reference |
| FPaprART | TTGACCATGACAGCGAGTGT | ([Bansal et al., 2017](#_ENREF_2)) |
| RPaprART | TTGGACAGAAATGCAGGATG | ([Bansal et al., 2017](#_ENREF_2)) |
| FPcrpRT | ATCATCATCTCGGGGAAGGT | This study |
| RPcrpRT | CAGCTGTTCGGAGATTTCG | This study |
| FPcmrRT | ATTGGCCGAAACGTTACAAG | This study |
| RPcmrRT | ACCATCGGCATCTCCAGTAG | This study |
| FPicl1RT | GCTTCTACCGCACCAAGAAC | This study |
| RPicl1RT | TCGAGGTGCTTTTTCCAGTT | This study |
| FPphoPRT | GCCTCAAGTTCCAGGGCTTT | This study |
| RPphoPRT | CCGGGCCCGATCCA | This study |
| FPumaART | CGTTATGCGGCATTCTTTG | This study |
| RPumaART | TGCGCAAATTTGAAGATGTC | This study |
| FPwhiB1RT | CACAAGGCGGTCTGTCGT | This study |
| RPwhiB1RT | GAGTCCTGGCCGGTATTCAG | This study |
| FPrpoBRT | GGAGGCGATCACACCGCAGACGTT | This study |
| RPrpoBRT | CCTCCAGCCCGGCACGCTCACGT | This study |
| FP16SrDNART | CTGAGATACGGCCCAGACTC | This study |
| RP16SrDNART | CGTCGATGGTGAAAGAGGTT | This study |
| Primersb |  |  |
| FPespAup | CGTGATCTTGATACGGCTCG | ([Anil Kumar et al., 2016](#_ENREF_1)) |
| RPespAup | GTTGTTGGTACCCTCGGCAAGATCGGC | ([Anil Kumar et al., 2016](#_ENREF_1)) |
| FPgapdhup | GAGTAGGCATCAACGGGTTTG | This study |
| RPgapdhup | GTGCTGTTGTCGGTGATGTC | This study |
| FPicl1up | AATAATAAGCTTACCGGATCCGCA | This study |
| RPicl1up | AATAATGGTACCGTTCGTGTCC | This study |
| FP16SrDNAup | CTGAGATACGGCCCAGACTC | ([Singh et al., 2014](#_ENREF_9)) |
| RP16SrDNAup | CGTCGATGGTGAAAGAGGTT | ([Singh et al., 2014](#_ENREF_9)) |
| FPsucCup | GGCTGTGATTGTGAGTTGGA | This study |
| RPsucCup | GCGAATAACTCCTTGGCTTG | This study |
| FPumaAup | TGTTGCTGCGTATGGTTGAG | This study |
| RPumaAup | AATCGATTGCGACTCTTCGT | This study |
| FPwhiB1up1 | AATAATAAGCTTAGATGTGATGGG | This study |
| RPwhiB1up1 | AATAATGGTACCAGCGCGTGGGCT | This study |

aFP, forward primer; RP, reverse primer

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