Table S2. Primers used for cloning, site-directed mutagenesis, sequencing and screening of transductants

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| Primer sequence |
| *gyrA* and *gyrB* cloning primersa, b |
| gyrA USF, 5'-GCGGTACCTTGTTCTCCATCCTGATGGG-3' |
| gyrA USR, 5'-GCGTCTAGATAGCTGCCCGATTCCTCCTC-3' |
| gyrA DSF, 5'-GCGAAGCTTAGGAGGAATCGGGCAGCTAG-3' |
| gyrA DSR, 5'-GCGACTAGTCGACCGTGATCATCCAGAC-3' |
| gyrB, USF, 5'-GCGGTACCGGGACGCACCAGGAAGAAAG-3' |
| gyrB, USR, 5'-GCGTCTAGAGCAGGGTTGCGTTAGACATC-3' |
| gyrB, DSF, 5'-GCGAAGCTTGATGTCTAACGCAACCCTG-3' |
| gyrB, DSR, 5'-GCGACTAGTAATCCTCTTCTACCTCAACAAC-3' |
|  |
| Site-directed mutagenesis primersc |
| A74S-F, 5'-CCACGCCAAGTCG**AGC**CGGTCGGTTGCC-3' |
| A74S-R, 5'-GGCAACCGACCG**GCT**CGACTTGGCGTGG-3' |
| A90G-F, 5'-CGCACGGCGAC**GGG**TCGATCTACGAC-3' |
| A90G-R, 5'-GTCGTAGATCGA**CCC**GTCGCCGTGCG-3' |
| R485C-F, 5'-GCTGGCCGATTGC**TGT**TCCACGGATCC-3' |
| R485C-R, 5'-GGATCCGTGGA**ACA**GCAATCGGCCAGC-3' |
| D500A-F, 5'-TCGTAGAAGGT**GCC**TCGGCCGGCGG-3' |
| D500A-R, 5'-CCGCCGGCCGA**GGC**ACCTTCTACGA-3' |
| D533A-F, 5'-GAAAGCGCGCATC**GCC**CGGGTGCTAAAGA-3' |
| D533A-R, 5'-TCTTTAGCACCCG**GGC**GATGCGCGCTTTC-3' |
| T539N-F, 5'-GACCGGGTGCTAAAGAAC**AAC**GAAGTTCAGGCG-3' |
| T539N-R, 5'-CGCCTGAACTTC**GTT**GTTCTTTAGCACCCGGTC-3' |
| T539P-F, 5'-CGGGTGCTAAAGAA**CCC**CGAAGTTCAGGCGA-3' |
| T539P-R, 5'-TCGCCTGAACTTC**GGG**GTTCTTTAGCACCCG-3' |
| E540D-F, 5'-GTGCTAAAGAACACC**GAT**GTTCAGGCGATCATCAC-3' |
| E540D-R, 5'-GTGATGATCGCCTGAAC**ATC**GGTGTTCTTTAGCAC-3' |
| E540V-F, 5'-GGTGCTAAAGAACACC**GTA**GTTCAGGCGATCATCA-3' |
| E540V-R, 5'-TGATGATCGCCTGAAC**TAC**GGTGTTCTTTAGCACC-3' |
| A543T-F, 5'-AACACCGAAGTTCAG**ACG**ATCATCACGGCGC-3' |
| A543T-R, 5'-GCGCCGTGATGAT**CGT**CTGAACTTCGGTGTT-3' |
| A543V-F, 5'-CACCGAAGTTCAG**GTG**ATCATCACGGCGC-3' |
| A543V-R, 5'-GCGCCGTGATGAT**CAC**CTGAACTTCGGTG-3' |
|  |
| *gyrA* and *gyrB* ORF sequencing primers |
| GyrA1F, 5'-TGGATGTCTAACGCAACCCTG-3' |
| GyrA1R, 5'-TTCTCCAGCGCCCAGAACAC-3' |
| GyrA2F, 5'-AATATCCCGCCGCACAAC-3' |
| GyrA2R, 5'-CAGTGCAATGACCTCGTCC-3' |
| GyrA3F, 5'-GGACGAGGTCATTGCACTG-3' |
| GyrA3R, 5'-AGCCGCGAATCTGGATGAC-3' |
| GyrA4F, 5'-ACCTGTTAGCCTTCCAGC-3' |
| GyrA4R, 5'-TTAATTGCCCGTCTGGTC-3' |
| GyrB1F, 5'-CCTACGGATAACACGTCGATC-3' |
| GyrB1R, 5'-TGAGACCACTCGTACCCGTC-3' |
| GyrB2F, 5'-GGTTAACGCGCTATCCAC-3' |
| GyrB2R, 5'-TGTTCACCACCGACGTCAG-3' |
| GyrB3F, 5'-CCAACACCATCAACACCC-3' |
| GyrB3R, 5'-TCGAACTCGTCGTGGATC-3' |
| GyrB4F, 5'-GACCGGGTGCTAAAGAAC-3' |
| GyrB4R, 5'-TTAGACATCCAGGAACCGAAC-3' |
|  |
| Screening primers |
| gyrA1F, 5'-TGGATGTCTAACGCAACCCTG-3' |
| gyrA1R, 5'-TTCTCCAGCGCCCAGAACAC-3' |
| gyrB2F, 5'-GGTTAACGCGCTATCCAC-3' |
| gyrB2R, 5'-TGTTCACCACCGACGTCAG-3' |
| gyrBS-F, 5'-GTGAACAAGGCTGTGTCC-3' |
| gyrBS-R, 5'-CACTTGAGTTTGTACAGCG-3' |
| Hyg-US, 5'-CAGGAATTCTGGGAGCCG-3' |
| Hyg-DS, 5'-AGTCGTGCAGGAAGGTGAAGG-3' |

aDesigned based on the sequence of H37Rv [[14](#_ENREF_14)], brestriction enzyme recognition sequences underlined, cthe nucleotide changes in the primers to allow A74S, A90G, R485C, D500A, D533A, T539N, T539P, E540D, E540V, A543T and A543V amino acid changes are indicated in bold.