

Mutation Analysis of Mycobacterial *rpoB* Genes and Rifampin Resistance Using Recombinant *Mycobacterium smegmatis*

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Rifampin is a major drug used to treat leprosy and tuberculosis. The rifampin resistance of *Mycobacterium leprae* and *Mycobacterium tuberculosis* results from a mutation in the *rpoB* gene, encoding the β subunit of RNA polymerase. A method for the molecular determination of rifampin resistance in these two mycobacteria would be clinically valuable, but the relationship between the mutations and susceptibility to rifampin must be clarified before its use. Analyses of mutations responsible for rifampin resistance using clinical isolates present some limitations. Each clinical isolate has its own genetic variations in some loci other than *rpoB*, which might affect rifampin susceptibility. For this study, we constructed recombinant strains of *Mycobacterium smegmatis* carrying the *M. leprae* or *M. tuberculosis rpoB* gene with or without mutation and disrupted their own *rpoB* genes on the chromosome. The rifampin and rifabutin susceptibilities of the recombinant bacteria were measured to examine the influence of the mutations. The results confirmed that several mutations detected in clinical isolates of these two pathogenic mycobacteria can confer rifampin resistance, but they also suggested that some mutations detected in *M. leprae* isolates or rifampin-resistant *M. tuberculosis* isolates are not involved in rifampin resistance.

eprosy and tuberculosis persist as important global public health concerns. Rifampin, a major drug used to treat these two infectious diseases, has a molecular mechanism of activity involving the inhibition of DNA-dependent RNA polymerase (15). In Escherichia coli, this enzyme is a complex oligomer comprised of four subunits, α , β , β' , and σ , encoded by *rpoA*, *rpoB*, *rpoC*, and *rpoD*, respectively. Rifampin binds to the β subunit of RNA polymerase and results in transcription inhibition (15). Mutations in the *rpoB* gene, encoding the β subunit of RNA polymerase, reportedly result in resistance to rifampin in several mycobacterial species, including Mycobacterium leprae and Mycobacterium tuberculosis (9, 21). The former has not yet been cultured on artificial media; it requires 11 to 14 days to double in experimentally infected mice. Therefore, it is difficult to determine the rifampin susceptibilities of M. leprae isolates. The standardized method using a mouse footpad takes more than half a year to determine the rifampin susceptibility of *M. leprae* isolates and requires 5×10^3 M. leprae bacilli (3), which require almost a year to prepare. In vitro drug susceptibility testing for M. leprae using a radioactive reagent requires more (107) M. leprae cells (7). In contrast, mutations in the *rpoB* gene of *M*. *leprae* can be detected in a few days or less. It would be very helpful if mutations responsible for rifampin resistance could be determined without performing mouse footpad testing. The main mutations that confer rifampin resistance to *M. tuberculosis* are located in the 81-bp core region of the *rpoB* gene, encompassing codons 507 to 533, known as the rifampin resistance-determining region (RRDR) (17, 18). About 95% of rifampin-resistant M. tuberculosis strains have a mutation in this region (18, 20). Four mutations, D516V, H526Y, H526D, and S531L, are most commonly associated with the high-level rifampin resistance of M. tuberculosis strains (4, 10, 19), but some other mutations in the 81-bp region have not yet been confirmed completely as being responsible for rifampin resistance.

We have established a method to determine the mutations responsible for the dapsone resistance of *M. leprae* using recombinant *Mycobacterium smegmatis* strains (16). In the present study, we assessed the applicability of the determination of rifampin resistance for analysis. We then analyzed *rpoB* mutations conferring rifampin resistance to *M. leprae* and *M. tuberculosis*.

MATERIALS AND METHODS

Bacterial strains and plasmids. *E. coli* DH5 α was used for DNA cloning. *M. smegmatis* mc²155 was used as a mycobacterial host to produce strains for drug susceptibility testing. Plasmids pYUB854 and phAE87 were kindly provided by W. R. Jacobs, Jr. (Department of Microbiology and Immunology, Albert Einstein College of Medicine, New York, NY). *M. smegmatis* mc²155 and its transformants were grown in Middlebrook 7H9 medium (Difco Laboratories, Detroit, MI) supplemented with 0.5% bovine serum albumin (fraction V), 0.2% glucose, 0.085% NaCl, 0.2% glycerol, and 0.1% Tween 80.

Site-directed mutagenesis. The wild-type *rpoB* genes of *M. leprae* and *M. tuberculosis* were amplified from *M. leprae* Thai-53 and *M. tuberculosis* H37Rv by PCR and cloned into pMV261. Site-directed mutagenesis was performed by using PCR with DNA polymerase (Takara PrimeStar HS; Takara Bio Inc., Kyoto, Japan) and the primers presented in Table 1. PCR products were purified and phosphorylated with T4 kinase and ATP and were then ligated to make them circular. The ligation mixture was used to transform *E. coli* DH5 α cells, and kanamycin-resistant colonies were isolated. Plasmids were extracted from the transformants. The mutated sequences were then confirmed by sequencing. The inserts of the plasmids were also cloned into pNN301 (16). Mutations introduced into the *M. leprae* rpoB or *M. tuberculosis* rpoB gene are listed in Table 2.

Disruption of the *rpoB* gene on the *M. smegmatis* chromosome. *M. smegmatis* mc²155 cells were transformed with plasmids carrying the *M. leprae* or *M. tuberculosis rpoB* gene with or without a point mutation. Recombinants were selected on LB medium containing kanamycin. Allel-

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TABLE 1 Primers used for this study

M. sergenetic MSBRUE GCTTAAGCAGGACGAGGACGAGGACGAGGCCAG MSBRUE GCCTTAAGCAGGAGAAGGACGAGGCCGGCAG rpd disruption, sporteam forward rpd disruption, sporteam forward rpd disruption, downstream forward REMOVE MIRBWTF GCGGGACGCGGGGCGGGAGGAGGACGCACCT MIRBWTFP GCGGGACGCCGGGACGGAGGCGGCGT MIRBWTFP GCGGCAGCCGGCGGCGCGGCGGCG MIRBWTFP GCGCAGCCGGCGGCGCGCGGCGGCG MIRBWTFP GCGCAGCCGGCGGCGCGCGGCGGCG MIRBWTFP GCGCAGCCGGCGGCGCGCGCGGCGGCG MIRBWTFP GCGCAGCCGGCGGCGCGCGCGGCGGCG MIRBWTFP GCGCAGCCGGCGGCGCGCGCGGCGGCG MIRBWTFP GCGCAGCCGGCGGCGCGCGCGCGGCGGCG MIRBWTFP GCGCAGCCGGCGGCGCGCGCGCGGCGGCGGCG MIRBWTFP GCGCAGCCGGCGGCGCGCGCGCGCGCGGCG MIRBWTFP GCGCAGCCGGCGGCGCGCGCGCGCGCGCGCG MIRBWSTGC GGACAGCCGGCGGCGCGCGCGCGCGCGCGCGCG MIRBWSTGC GCGACAGCCGGCGGCGCGCGCGCGCGCGCGCGCGCGCGC	Primer	Sequence ^a	Application
MARGUP CCCTIANGGAAGGAAGACGAGCCAC rpd disruption, gustrem forward MSBUP CCCTIANGAAGAAGACGACTCTCTCCAGC rpd disruption, gustrem reverse MSBUP CCAMCETTCCCCAAC Detection of rpd disruption, downstrem reverse MSBUP CCAMCETTCCCCAAC Detection of rpd disruption, downstrem reverse MSBUP CCTICAGCAGTCCCCGCGCCAACGATCCCCCC rpd disruption, downstrem reverse MSBUP CCTICAGCAGTCCCCGCGCGCACCACCCCC Detection of rpd disruption, reverse MSBUR CCTICAGCCGCGCGCGCGGGGTCTT Introduction of point mutation accdons 507 and 508 MLRBWTR CCCGCGCCGCGGCGGGGGTCTT Introduction of point mutation accdons 517 and 508 MLRBWTR CCGCACCTCGCTCGCCAACGAAT Introduction of point mutation accdons 518, and 517 MLRBWTRI CCGCACCTGGCTCGCAACGAAT Introduction of point mutation accdons 518, and 533 MLRBWTRI CCGCACCTGGCTCGCAACGAAT Introduction of point mutation accdons 513, 532, and 533 MLRBWTRI CCGCACCTGGCTCGCAACGAAT Introduction of point mutation CGCSP-4GC MLRBWTRI CCGCACCTGGCTCGCAACGAAT Introduction of point mutation CGCSP-4GC MLRBWTRI CCGCACCTGGCTCGCACGACGCTC Introduction of point mutation CGCSP-4GC	M smeamatis	1	11
MSBRIT CCTCLEAGEAGATCCATCCTTCCACCA rpd Bamplion, gesteram reverse MSBRUR CCACLIANTACCCACCACCACCACCACCACCACCTTCTCG rpd Bamplion, gesteram reverse MSBR CCTTCTGACCATCACCCCCCCCCCCCCCCCCCCCCCCCC	MSRBUF	GCCTTAAGGAGGAGAAGGACGAGGCCAC	rboB disruption upstream forward
MSRDP CCARCCTTTCGCCCACCAATCGCCCCTT rpd 8 struption, downstream toward MSRDF TGGTCAAGCAATCGCCGCCCCTTC rpd 8 struption, downstream toward MSRDF TGGTCAAGCAATCGCCCCCCCC Detection of rpd disruption, forward MSRDF CGGTGTGTGTGCGCGAAGCATCTTCTATGC Detection of rpd disruption, reverse MIRWTP CGGGATCGTGTGCGCAAGCATCTTCTATGC Coming of M lepror rpd, forward MIRWTP CGGGGTGCGCGAAGCATCTTCTATGC Introduction of point matrian acidems 307 and 508 MIRWTP CGGTGCGCGTGGTGCCCAAGCATCT Introduction of point matrian acidems 307 MIRWTP CGGCAGCGGTGTGGTGCCGAAGCAT Introduction of point matrian acidems 305, and 371 MIRWTR CGGCAGCGGTGTGGTCCCAAGCAAT Introduction of point matrian acidems 305, and 333 MIRBWTR CGGCAGCGGGTGTGTGCCCAAGCAAT Introduction of point matrian acidems 305, and 333 MIRBWTR CGGCAGCGGGTGGTGTCCCCAAGCACT Introduction of point matrian acidems 305, and 333 MIRBWTR CGGCAGCGGGTGGTGTGCCCAAGCACTC Introduction of point matrian acidems 307, 305, and 371 MIRBWTR CGGCAGCGGGTGGTGTT Introduction of point matrian CGGSDAGC MIRBWTR CGGCGCGGCGGTGGGTTT Introduction of point matrian CGGSDAGC MIRBWTR CGGTGGCGCGGGGGGGGTTT	MSRBUR	GCTCTAGACAAGATGCATCCTTCCAGCA	rpoB disruption, upstream reverse
MSRBPR GCALLACTACCCACCCACCTCTCTC ipped alamption, downartourn reverse MSRR COTTOTTGACCATCGATCCTCC Detection of ped disruption, forward Market GCGATLACTGCTGCGAGGAGTCCTTCT Change of M lprane rpsR, forward MIRINFT GCGATLACCTGCTGCGAGGAGTCTTCTCTACG Change of M lprane rpsR, forward MIRINFT GCGATLACCGCTGGCGCGGAGGGTT Introduction of point mutation and coden 350 MIRINFT TGCGGCGCGGGCGGGGGGTT Introduction of point mutation and coden 351, 516, and 517 MIRINFTS TGCGGCGCGGGCGGGGGGTT Introduction of point mutation accdens 351, 516, and 517 MIRINFTR CGCGGCGCGGGGGGGTGTGGGGGGGGGGTT Introduction of point mutation accdens 353, 516, and 517 MIRINFTR CGCGGCGCGGGGGGGTGTGGGGGGGGGGGGGGGGGGGG	MSRBDF	GCAAGCTTTCGCGCAACGAATCCGCGTC	<i>rpoB</i> disruption, downstream forward
MSRBF TGGTCAAGCATTCCTCAAC Desction of pab discuption, forward MSRBF CGTGTGTGGACGATCCTCG Desction of pab discuption, forward MIRWTF CGCGATCCGTGCTGGACGATCCTTCTAGG Cloning of M. leptor pab, forward MIRWTF CGCGATCCGTGCGGACGATCCTTCG Introduction of point mutation at colons 50 and 500 MIRWTF CGCGACCGTGGTGGTGCCGAAGAAT Introduction of point mutation at colons 51, 51, 51, and 517 MIRBWTRI CGCACGGCGCTGTGGTGCCGAAGAAT Introduction of point mutation at colons 51, 51, 51, and 517 MIRBWTRI CGCACGCTGCTGGTGCCCAAGAAAT Introduction of point mutation at colons 531, 535, and 533 MIRBWTRI CGCACGCTGCTGGTGCCCAAGAAAT Introduction of point mutation at colons 531, 535, and 533 MIRBSIGG CGAACACGTGCGTGTGGCCAAGAAT Introduction of point mutation at colons 531, 532, and 533 MIRBSIGGT CGCACGCGTGGTGTGTGCCAAGAAT Introduction of point mutation at colons 50, 563, 563, 573, 764, 764, 774 MIRBSIGGT CGCGACGCGTGGTGTGGTGCCAAGAAT Introduction of point mutation CCG31CAT MIRBSIGGT CGCGCACGCTGGTGCTGAGGCCCGGAGGTT Introduction of point mutation CCG31CAT MIRBSIGGT CGCGCACGCTGGGCCCGGGCGGTTT Introduction of point mutation CCG31TGG MIRBSIGG	MSRBDR	GCACTAGTAGCGCACGCAGCTTCTTCTG	<i>rpoB</i> disruption, downstream reverse
MSRR CGTTGTTGACGATGATCTCG Detection of pol disruption, reverse AL lprac MLREWTF GCGTTGTGTGTGGTGGTGGTGGTGGTGGTGTGTGTGTGGTG	MSRBF	TGGTCAAGCAGTTCCTCAAC	Detection of <i>rpoB</i> disruption, forward
M Jayner MIRBWITE GCGETALACCTAGCCAGATCCTATGG Chaning of M. Jayner public problems Chaning of M. Jayner public problems MIRBWITE GCGTLAACCTAGCCAGATCCTTCTATGG MIRBWITE CAGTTCATGGATCAGAACCAGTCGTT Introduction of point mutation at codom 507 and 508 MIRBWITE CGGCAGCTGGTGGTGGCCAAGAAT Introduction of point mutation at codom 517 and 538 MIRBWITE CGGCAGCTGGTGGTGCCAAGAAT Introduction of point mutation of GCS97-AGG MIRBWITE CGGCAGCTGGTGGTGCCAAGAAT Introduction of point mutation GGCS97-AGG MIRBWITE CGGCAGCTGGTGGTGCCAAGAAT Introduction of point mutation GGCS97-AGG MIRBWITE CGGCAGCGTGTGGTGCCCAAGAAT Introduction of point mutation GGCS97-AGG MIRBWITE CGGCAGCGTGTGGTGCCCAAGAAT MIRBWITE CGGCAGCGTGGTGGTGTGTGTGAAGAAT MIRBWITE TGTGGGCCCGGGCCCGGGTGTTT MIRBWITE TGTGGGCCCGGGCCCGGGGTGTTT MIRBWITE TGTGGGCCCGGGCCGGGTGGTTT MIRBWITE TGTGGGCCCGGGGCCGGGTGTTT MIRBWITE TGTGGGCCCGGGGCCGGGGTGTT MIRBWITE CGTGCAGCCTGGGGCCCGGGGGTGTT MIRBWITE GGGTGCAGCGTG	MSRBR	CGTTGTTGACGATGATCTCG	Detection of <i>rpoB</i> disruption, reverse
a. main GCGLIATCCTCCGCAGAGCATCTT Claning of AL Japac public forward MLREWTF GCGLIALCCTAGCAGCACATCTTCTATGG Claning of AL Japac public forward MLREWTF CGGLIALCCTAGCAGCACATCTTCTATGG Claning of AL Japac public forward MLREWTF TGTGGCGGCGGCGGGTGGTT Introduction of point mutation at codon 526 MLREWTR GCGCGCGCGCTGGTGGTCGGTCGGTG Introduction of point mutation at codon 531, 512, and 513 MLRESTGGC GCACACGTCGCTGGTGCGCAGAGAAT Introduction of point mutation GCG397GGC MLRESTGGC CGACACGTCGCTGGTGCGCGCAGAAAT Introduction of point mutation GCG397GGC MLRESTGG CGTGTCATGGATCAGAACACCCTC Introduction of point mutation GCG39AGC MLRESTCAT CGGTGGCCGCGGCGCGGGGGTGTT Introduction of point mutation CAS31TGG MLRESTCAT CGGTGGCCGCGGCGGGGGTGTT Introduction of point mutation CAS31TGG MLRESTCAT CGGTGCGCCGGGCGGGGGGTGTT Introduction of point mutation CAS31TGG MLRESTACC CGGCGCGCCGGGCGGGGGTGTT Introduction of point mutation CCS31TGG MLRESTACC CGGCGGCGCGGGGGGTGTT Introduction of point mutation CCS31TGG MLRESTACC CGGCGGCGCGGGGCGGGGGGTT Introduction of point mutation CCS39TGG <td>Mlapras</td> <td></td> <td></td>	Mlapras		
MLERWYTR CGETTAMCCTAAGCCAGATCTTCTATGG Cloning of M. Lepar. (pB, revers) MLERWYTP: CAGTCAGGGCCAGGACAGCCCC Introduction of point mutation at coden 526 MLERWYTP: TTGCGGCCTGGGCCCGGTGGCTC Introduction of point mutation at coden 547 MLERWYTP: CGGCGCCTGGGCCCGGATGGCCCCG Introduction of point mutation at coden 547 MLERWYTP: CGGCGCCTGGTGCCGCAGAGAT Introduction of point mutation at coden 531, 516, and 517 MLERWTR: CGGCAGCTGGTGCGCGCGGAGAAT Introduction of point mutation GCGC9GGG MLERBWTRAC CGACAGCTGGCTGCTGCGCAGAGAT Introduction of point mutation CGC30GGG MLERBWTRAC CGACAGCTGGCGCGTGGTGCGCGGAGAAT Introduction of point mutation CGC30GGG MLERBWTRAC CGACAGCTGGTGCGCCGGGGCGCGGGTT Introduction of point mutation CGC30CAT MLERBWTRAC CGGCGCGCGGGGCCCGGGGTGTT Introduction of point mutation CGC30CAT MLERBWTRACG TGGCGGCCGGGGGCCCGGGGTGTTT Introduction of point mutation CGC30CAT MLERBWTRACG TGGCGGCCGGGGCCCGGGGTGTTT Introduction of point mutation CGC30CAT MLERBWTRACG TGGCGGCCGGGGCCCGGGGGTGTTT Introduction of point mutation CGC30CAT MLERBWTR CGGGGCCGTGGGGCCCGGGGGGCGCC I	MI RBWTF	CCCCATCCCTCCAACCATCCATCTT	Cloping of M letragerbaB forward
MLRBWTFI CAGTTCATGGATCAGAACAACCCTC Introduction of point mutation at coden 507 and 508 MLRBWTFIS TGCGACTCAGCCGGGGTGGTT Introduction of point mutation at coden 547 MLRBWTFIE CGCACGCGGCGGTGGTGCGCGAACTCGCCCG Introduction of point mutation at coden 531, 553, and 533 MLRBWTRI2 CGCGGGGCTGTGGGCGCGAACT Introduction of point mutation at coden 531, 553, and 533 MLRBWTRI2 CGCACACTGGCGCGTCGTGCGAACAAT Introduction of point mutation ACC307 =>AGA MLRBWSTAGC CGACAGCTGGCTGCTGCGAACAACCTC Introduction of point mutation ACC308 =>AGA MLRBWSTAGC CGACAGCTGGCTGCTGAGGACCAACAACCTCC Introduction of point mutation ACC308 =>AGA MLRBWSTAGC CGACAGCTGGCTGCTGAGGCCCGGGCCGGTT Introduction of point mutation ACC308 =>AGA MLRBWSTAGC CGCAGCGCGCGCCCGCCGCGGGTGTTT Introduction of point mutation ACC308 =>AGA MLRBWSTAGC CGCAGCGCGCGCCCGCCGCGGGTGTTT Introduction of point mutation ACC308 =>AGA MLRBWSTAGC TGTCGCGCGGGCCCGCGCCGGGTGTTT Introduction of point mutation ACC308 =>AGA MLRBWSTAGC TGTCGCGCGGGCCCGGCGCGGGTTT Introduction of point mutation ACC308 =>AGA MLRBWSTAGC TGTCGCGCGGGCCCGGCGCGGGTGTTT Introduction of point mutation ACC308 =>AGA MLRBWS	MIRBWTR	GCGTTAACCTAAGCCAGATCTTCTATGG	Cloning of M. leprae rboB, reverse
MLRBWTP2 TGTCGGCCTGGGCCCGGGTGGTT Introduction of point mutation at coden 526 MLRBWTP1 CGCACAGCTGGCCGAGTGTGCGCGAGAGT Introduction of point mutation at coden 513, 516, als 517 MLRBWTP1 CGCACGCTGGTGGTGCCGAAGAAT Introduction of point mutation at coden 513, 516, als 517 MLRBWTP2 CGCACGCTGGTGGTGCGGAAGAAT Introduction of point mutation at Coden 513, 556, als 517 MLRBBTAGC CGCACAGCTGGCTGGTGGCGAAGAAT Introduction of point mutation at CCS08AGC MLRBSTAGC CGCACAGCTGGCTGGTGGCGAAGAAT Introduction of point mutation at CCS08AGC MLRBSTGCA CGCACAGCTGGGTGTGGTGGGGAAGAAT Introduction of point mutation at CCS08AGC MLRBSTGCA CGCACAGCTGGGTGGTGGTGGGGAAGAAT Introduction of point mutation at CCS08AGC MLRBSTGCA CGCACAGCTGGGCGGGGGGTTT Introduction of point mutation at CCS28TAC MLRBSTGCA CGCAGCGGCGCGGCGGGGGGTTT Introduction of point mutation at CCS33TGG MLRBSTGCG TGTCGGGCCCAGGGCCGGGGGGTTT Introduction of point mutation at Coden 507, 508, 510, 511, 512, and MLRBSTGCG TGTCGTGGCCGGGGCGCGGGGGGTT Introduction of point mutation at codens 507, 508, 510, 511, 512, and MTRBWTP1 CATTCATGGGCCGGGGCGGGGGGGGGGGGGGGGGGGTT Introduction of point mutation at codens 5	MLRBWTF1		Introduction of point mutation at codons 507 and 508
MLRBWTFB TTCGCACTACGOCCGATGTGCCCCC Introduction of point mutation at codons 31, 354, and 517 MLRBWTR2 CCGGCGCGCTGTGGGCAGAGAT Introduction of point mutation at codons 31, 354, and 533 MLRBWTR2 CCGGCGCGCGCGGCGGCGGCAGAT Introduction of point mutation at CCG307-AGG MLRBW07AGC CGACAGCTGGCTGGTGCGAAGAAT Introduction of point mutation ACC508AGA MLRB807AGC CGACAGCTGGCTGCGACGAAT Introduction of point mutation ACC508AGA MLRB813GTG GTCTTCATGGACTCAGACAACCTC Introduction of point mutation CAC530GAC MLRB817CAT CACGTCATGAGCACGGACCCTC Introduction of point mutation CAC530GAC MLRB817GG TGTCGAGCCGGGCCCGGCGCGGTTT Introduction of point mutation CAC530GAC MLRB8317GG TGTCGCGCGGGCCGGGCGCGGTTT Introduction of point mutation CAC530GCG MLRB8317GG TGTCGCGCGGCGCCGGCGGGTTT Introduction of point mutation CC533GCG MLRB8317CG TGTCGTGCCGGGCCCGGCGGGTTT Introduction of point mutation CC530GCG MLRB8317CG TGTCGTGCCGGGCCCGGCGGGGTTT Introduction of point mutation CC530GCG MLRB8317CG TGTCGTGGCCGGGCCCGGCGGGGTT Introduction of point mutation CC530GCG MLRB8317CC GGGTCAACGCGGGCCGGGGGGGTC <td< td=""><td>MLRBWTF2</td><td>TGTCGGCGCTGGGCCCGGGTGGTTT</td><td>Introduction of point mutation at codon 526</td></td<>	MLRBWTF2	TGTCGGCGCTGGGCCCGGGTGGTTT	Introduction of point mutation at codon 526
MLRBWTEI CGACACCTOCCTOGCCAGAGAT Introduction of point mutation at codons \$13, 516, and 317 MLRBWTGGG CGACACCTOGCTOGTCAGCCCGA Introduction of point mutation at codons \$13, 516, and 333 MLRBWTGGG CGACACCTOGCTOGTCAGAGAAT Introduction of point mutation at codons \$13, 516, and 333 MLRBWTGG CGACACCTOGCTOGTCAGAAGAAT Introduction of point mutation CACS98->ACC MLRBBSIGAT CGGTCATGTGAGACAACCCTC Introduction of point mutation CACS98->ACA MLRBSSTAT CGGTTCATGGATATACAACAACCCTC Introduction of point mutation CAS31->CTG MLRBSSTAT CGGTCAGCTGGGCCCGGGTGTTT Introduction of point mutation CAS31->CTG MLRBSSTATG GCGCGGCGTGTGTAGCGAGAGCCGGTTT Introduction of point mutation CGS31->TTG MLRBSSTATG GCGCGCGCGTCAGCGCGGTGTTT Introduction of point mutation CGS31->TGG MLRBSSTATG GCGTCAGCCAGACACTCCCCCCCCGCG Introduction of point mutation CGS32->TGG MLRBSSTATG GCGTCAGCTGAGCCAGACATTCCCCCCCCCCGC Introduction of point mutation CGS32->TGG MLRBWTF GCGAGATTCCTGCGCCAGAGC Cloning of M. tubercalosis tpp8, frowad MTRBWTF GCGGCGCTGTGGCGCGGGCGCGGCGCC Introduction of point mutation at codons 50, 598, 510, 511, 512, and MTRBWTR1	MLRBWTF3	TTCGCACTACGGCCGGATGTGCCCG	Introduction of point mutation at codon 547
MIRRNTP2 GCCGCGCCTTCTGGGTCGGCCCGAA Inroduction of point mutation GCGSO*-AGG MIRRS07AGC CGACAGCTGGCTGGTCCGAAGAAT Inroduction of point mutation GCGSO*-AGG MIRRS07AGC CGACAGCTGGCTGTCCGAAGAAT Inroduction of point mutation GCGSO*-AGG MIRRS07AGC CGACAGCTGGCTGTCGCGAAGAAAT Inroduction of point mutation CAGS13GTG MIRRS16AT CGTTCATGGATCAAACAACCTC Inroduction of point mutation CAGS17CAT MIRRS17CAT CAGTTCATGGATCATAACAACCACCTC Inroduction of point mutation CAGS17CAT MIRRS17CAT CAGTTCATGGATCATAACAACCACCTC Inroduction of point mutation CAGS17CAT MIRRS17CAT CGCGGCGCTTGTTAGCTCAGGCCCGGGTGTTT Inroduction of point mutation CGS31TGC MIRRS17CAT TGTGGCACCTGGGCCCGGGTGGTTT Inroduction of point mutation CGS31TGG MIRRS17CG TGTGGCACCCGGGGCCGGGGTGGTTT Inroduction of point mutation GCS31TGG MIRRS17CG TGTGGGCACGCGGGGGTGGTTT Inroduction of point mutation GCS31TGG MIRRS17CG GGTGGCAACTCCTGGCCAAGAGC Cloning of M. Inherculosis rpoB, forward MIRRS17CG GGTGCGAACTCCTGGCCCAAGAGC Cloning of M. Inherculosis rpoB, forward MIRRS17C GGTCAGCTGGGGCGCCGAGGGGTC Inroduction of point mutation at c	MLRBWTR1	CGACAGCTGGCTGGTGCCGAAGAAT	Introduction of point mutation at codons 513, 516, and 517
MIRBS97GG CGACACCTGGCTGGTCCGAAGAT Inroduction of point mutation GGCS07GGG MIRBS98ACA CGACAGCTGGCTGTCCGAAGAAT Inroduction of point mutation GGCS07AGC MIRBS16AT CGACAGCTGGCTGTCCGAAGAAT Inroduction of point mutation GACS18AGA MIRBS16AT CAGTTCATGAACAACCACCTC Inroduction of point mutation GAS17CAT MIRBS16AT CAGTTCATGAACAACCACCTC Inroduction of point mutation GAS17CAT MIRBS17G CGCCGCGCTTGTGGCCCCGGGTGGTTT Inroduction of point mutation CAS31CAT MIRBS317G TGTCGGCCGTGGCCCCGGGTGGTTT Inroduction of point mutation TGCS31TGG MIRBS37CG TGTCGTCCCTGGCCCCGGGTGGTTT Inroduction of point mutation TGCS31TGG MIRBS37CG TGTCGTCCCTGGGCCCGGGCCGGGTGTT Inroduction of point mutation TGCS31TGG MIRBS37CG TGTCGTCGCTGGGCCCGGCGCGGGTT Inroduction of point mutation TGCS31TGG MIRBS37TC GGGTCACGTCACGACACCCCCGCACGC Cloning of M. tuberculois ppB, forward MIRBS7TP1 ATTCGTGGCGCTGGGCCGCGCGGTC Inroduction of point mutation at codons 507, 508, 510, 511, 512, and 513 MTRBWTP1 CGTCGGCGCTGGGCCCGACGGTC Inroduction of point mutation at codons 514, 518, 518, 519, 319, and 521, deltion MTRBWTP1 CGTCGGCGCTGGGCCGC	MLRBWTR2	GCCGGCGCTTGTGGGTCAGGCCCGA	Introduction of point mutation at codons 531, 532, and 533
MIRB807ACC CGAAACCTGGCTGTCCGAAGAAT Introduction of point mutation ACCS09ACC MIRB813GTG GTGTTCATGATCAGAACAACCCTC Introduction of point mutation ACGS19ACA MIRB813GTG GTGTTCATGATCAGAACAACCCTC Introduction of point mutation CAS13ATA MIRB81AC CAGTTCATGATCAGACAACCCTC Introduction of point mutation CAS13ATA MIRB831TGG CGCGCGCTGTGAGGCCCGGGTGGTTT Introduction of point mutation CAS13TGG MIRB831TGG TGTGGCGCGTGGGCCCGGGTGGTTT Introduction of point mutation TGS31TGG MIRB831TGG TGTGGCGCGGGGCGGGGTGGTTT Introduction of point mutation TGS31TGG MIRB831CG TGTGGCGCGGGGCGGGGTGGTTT Introduction of point mutation TGS31TGG MIRB87TE GGGTGCACGGCGGGGCGTGGTTT Introduction of point mutation TGS31TGG MIRB87TE GGGAATTCTTGGGCACGGCGCGC Introduction of point mutation TGS31ATC MIRB87TE GGGAATTCTTGGCAGATCCCGCCACAGC Cloning of <i>M. tuberculosis tpoB</i> , reverse MIRB87TE GGCTCAGCTGGGCCCGGCGGGC Introduction of point mutation stoons 507, 508, 510, 511, 512, and MIRB87TE TGGGGCCTGGGGTGGTGTGTCGCGAAGAA Introduction of point mutation at colons 512, 518, 519, and 521 MIRB87TE TGGGGCCTGGGGTGGTGTGTGCGCAAGAA Introduction of point mutation at colons 516, 582, and 531<	MLRB507GGG	CGACAGCTGGCTGGTCCCGAAGAAT	Introduction of point mutation GGC507→GGG
MLRB3508ACA CGACAGCTGGCTTGTGCCGAAGAAT Introduction of point mutation ACC308-ACA MLRB316AAT CAGTTCATGGATCAGAACAACCCTC Introduction of point mutation CAG319-GTG MLRB316AAT CAGTTCATGGATCAGAACAACCCTC Introduction of point mutation CAG319-CAT MLRB351TAC GCCGGGGCTTGTAGGCCCGGGTGGGTT Introduction of point mutation CG331-TTG MLRB331TG TGTGGGCGCTGGGCCCGGGTGGTTT Introduction of point mutation CG331-TTG MLRB331TG TGTGGGCGCGGGGCCGGGTGGTTT Introduction of point mutation CG331-TTG MLRB332TCG TGTCGGCGCTGGGCCCGGGTGGTTT Introduction of point mutation CTG331-CCG MLRB347ATC GGGTGCACGTCACGGATCCCCGCCGGTGGTTT Introduction of point mutation CTG331-CCG MTRBWTF GCGCACGTCACGAACTCCCCGCCGAGG Cloning of M. tuberculosis pol, forward MTRBWTF GCGTCGCGGGGCCCGGGCGGTC Introduction of point mutation at codons 507, 508, 510, 511, 512, and 513 and deletion of codons 514, and 513 and feleion of codons 514, and 514 and 513 and feleion of codons 514, and 514 and 513 and feleion of codons 514, and 514, deletion MTRBWTF CTGTCGGCGTGGGTCCCAGCGGGCGGGTGCGAGAA Introduction of point mutation GCG30-~AGT MTRBWTR GGCTCACGTGGCTGGGTCCCAGAGAA Introduction of point mutation GCG30-~AGT MTRBWTP CTGTCGGCGTGGGGCCCCGGGGGTGGTGGCGAGAA Introduction of point muta	MLRB507AGC	CGACAGCTGGCTGGTGCTGAAGAAT	Introduction of point mutation GGC507→AGC
MIRB313GTG GTGTTCATGGATCAGAACAACCCTC Introduction of point mutation CAG513GTG MIRB51AAT CAGTTCATGATAACAACCCTC Introduction of point mutation CAG513CAT MIRB51ACAT CAGTTCATGATAACAACCCTC Introduction of point mutation CAG513CAT MIRB531TG TGTGGGGCGTGGGCCCGGGTGGTTT Introduction of point mutation CAG531TTG MIRB531TG TGTGGGCGCGGGCCCGGGGTGTT Introduction of point mutation CG533TCG MIRB531TG TGTGGCCCGGGCCCGGGGTGTT Introduction of point mutation CG533CCG MIRB533CCG TGTCGGCCCGGGCCCGGGGGTGTT Introduction of point mutation CG533CCG MIRB533CCG TGTCGGCGCCGGGGCCCGGGGGGTT Introduction of point mutation CG53ATC MIRB533CCG TGTCGGCGCTGGGCCCGGCGCGC Cloning of M. tuberculosis ppb, forward MIRB547F GCGAATTCTTGGCAGACACCCGCACA Introduction of point mutation at codons 514, 516, 518, 519, 511, 512, and 513 and delion of codons 506-508 MTRBWTR GGCTCAGCTGGGCCGGGGCGCGACA Introduction of point mutation GC530AGC MTRBWTR GGCTCAGCTGGGTCGGAACAA Introduction of point mutation GC530AGC MTRBWTR GGCTCAGCTGGTGGTCGAAGAA Introduction of point mutation GC530AGC MTRBS07AG GGCTCAGCTGGTGGTCGAAGAA Introduction of point mutation GC530AGC <t< td=""><td>MLRB508ACA</td><td>CGACAGCTGGCTTGTGCCGAAGAAT</td><td>Introduction of point mutation ACC508→ACA</td></t<>	MLRB508ACA	CGACAGCTGGCTTGTGCCGAAGAAT	Introduction of point mutation ACC508→ACA
MIRBS16AAT CAGTTCATGAATCAACCCCC Introduction of point mutation CAGS10XAT MIRBS17AC CAGTTCATGGATCATAACAACCCCC Introduction of point mutation CAGS10XCT MIRBS31TG GCCGGCGCTTGATGAGTCAGGCCCGGA Introduction of point mutation CAGS10FTG MIRBS31TG TGTGGGCGCGGCCCGGGTGGTTT Introduction of point mutation CAGS30FTG MIRBS31TG TGTGGGCCGCGGCCCGGGTGGTTT Introduction of point mutation CAGS30FTG MIRBS47ATC GGTGCACGTCACGGGTCCCGGGGGTGGTT Introduction of point mutation GTG547ATC MIRBS47ATC GGGTGCACGTCACGGACTCCCCCCAGGG Cloning of M tuberculosis rpaB, reverse MTRBWTF GCGAATTCTTGGCACAGATCCCCCGACG Cloning of M tuberculosis rpaB, reverse MTRBWTF1 ATTCATGGACCAGAAACACCCCGAC Cloning of M tuberculosis rpaB, reverse MTRBWTF1 CTGTGGCGCTGGGGCCCGGCGGGTC Introduction of point mutation at codons 505-508, 510, 511, 512, and 511 and deltoin of codons 514 and 513 and deltoin of codons 714, 516, 518, 519, and 521, deltoin of codons 716, 746, 746, 746, 746, 746, 746, 746, 74	MLRB513GTG	GTGTTCATGGATCAGAACAACCCTC	Introduction of point mutation CAG513→GTG
MLRB317CAT CACHTCATGGATCATAACACCCTC Introduction of point mutation CG31-~CAT MLRB321TCG GCGGCGCTGTGGCCCGGGGCGCGGTTT Introduction of point mutation CG31-~TGG MLRB33TCG TGTGGCGCGGGGCCGGGGGGTTT Introduction of point mutation CG331-~TGG MLRB33TCG TGTGGCGCGGGGCCGGGGGGTTT Introduction of point mutation CG331-~TGG MLRB33TCG TGTGGCGCGGGCCGGGGGTGTT Introduction of point mutation CG331-~TGG MLRB37TATC GGGGCACGTCACGGAATTCCTGACCC Introduction of point mutation CTG34-~ATC MLRB37TATC GCGAATTCTTGGCAGATTCCCGCCAGAG Cloning of M. tuberculosis rp08, forward MTRBWTF GCGAAGTCTTGGCACGAAACCCCGCT Introduction of point mutation at codons 507, 508, 510, 511, 512, and MTRBWTF1 AATTCATCGGCGCTGGGCCCGGCGGCGTC Introduction of point mutation at codons 507, 508, 510, 511, 512, and MTRBWTR1 GCGCTAGCTGGCTGGTGCCGAGAA Introduction of point mutation at codons 514, and 513, and 511, deletion MTRB307GG GGCTCAGCTGGCTGGTGCGCGGGGTGGTGGAAAA Introduction of point mutation ACC508-~CAC MTRB307GG GGCTCAGCTGGCTGGTGGCGCGGAAGAA Introduction of point mutation ACC508-~CAC MTRB307AT GCGCTAGCTGGCTGGTGGCGCGAAGAA Introduction of point mutation ACC508-~CAC MTRB307AT GGCTCAGCTGGCTGGTGGCGCGGAAGAA <	MLRB516AAT	CAGTTCATGAATCAGAACAACCCTC	Introduction of point mutation GAT516→AAT
MLRBS26TAC GCCGGCGCTTGTGGGCCGGGGCCGG Introduction of point mutation CG231->TTG MLRBS3TGG TGTGGGCGGGGGCCGGGGGGTTT Introduction of point mutation CG31->TTG MLRBS3TGG TGTGGGCGGGGGCCGGGGGGTTT Introduction of point mutation CG331->TCG MLRBS3TATC GGTGGCCGGGGCCGGGGGTGTT Introduction of point mutation CG332->TCG MLRBS47ATC GGGTGGCCGGGGCGGGGCTC Introduction of point mutation CTC347->ATC MLRBS47ATC GCGAAGTTCTGGGCGCGGGCGC Introduction of point mutation CTC347->ATC MLRBS47ATC GCGAAGTTCTGGGGCCGGGCGCGC Introduction of point mutation CTC347->ATC MTRBWTF GCAAGCTTTTACGCAGAACACCCGCGT Introduction of point mutation at codons 514, 516, 518, 519, and 521, deletion of codons 514, 516, 518, 519, and 521, deletion of codon of 518, and insertion of TTC between codons 514, 516, 518, 519, and 521, deletion of codon 518, and insertion of TCC and TCG31->TTG MTRBWTR2 TCGGCGCTGGTGTGGGCCGAGAGAA Introduction of point mutation GC303->TCG and TCG31->TTG MTRBS90CAC GGCTCAAGTGGCTGGGTGCGGAGAAA Introduction of point mutation GC303->TCG and TCG31->TTG MTRBS90CAC GGCTCAGTGGCTGGGTGCGGAGAAA Introduction of point mutation GC30->GCAT MTRBS90CAC GGCTCAGTGGCTGGGTGCGCGAAGAA Introduction of point mutation CAC318->CAT MTRBS90CAC GGCTCAGTGGCTGGGTGCGCGAAGAA	MLRB517CAT	CAGTTCATGGATCATAACAACCCTC	Introduction of point mutation CAG517→CAT
MLRB331TG TGTGGCGCTGGCCCGGGGGGTGTT Introduction of point mutation TCG31→TTG MLRB33TCG TGTCGCGCCGGGGCCCGGGGGTGTT Introduction of point mutation CG331→TGG MLRB33TCG TGTCGCGCGCGGGGCCGGGGGTGTT Introduction of point mutation CG333→TCG MLRB3TTC GGGTGACGTCACGGACTCCAGGGTGTTT Introduction of point mutation CTG31→TCG MLRB3TTC GGGTGACGTCACGGACTCCCGCCAGAG Cloning of M. tuberculosis rp0B, forward MTRBWTF GCAAGTTTTGGCAGACTCCCGGCAGAC Cloning of M. tuberculosis rp0B, forward MTRBWTF1 AATTCATGGACGAGAACCCGGGGGTC Introduction of point mutation at codons 507, 508, 510, 511, 512, and MTRBWTF2 CTGTGGGGCGCGGGGGGGCC Introduction of point mutation at codons 514, 518, 519, and 521; deletion of codons 506, 508 MTRBWTR2 CTGTGGGGGTGGACACCCGGAGAA Introduction of point mutation at Codons 514, 518, 519, and 521; deletion of codons 504, and 515 MTRB907AGC GGCTCAGCTGGGTGGGTGCGAGAGAA Introduction of point mutation ACC508→CAC MTRB907AGC GGCTCAGCTGGCGGGGCGCGGAGAAA Introduction of point mutation ACC508→CAC MTRB907AGC GGCTCAGCTGGGTGGGCCCGGAGAAA Introduction of point mutation ACC508→CAC MTRB907AGC GGCTCAGCTGGGTGGGCCCGCAGAAAA Introduction of point mutation ACC508→CAC MTRB907AGT GGCTCAGCTGG	MLRB526TAC	GCCGGCGCTTGTAGGTCAGGCCCGA	Introduction of point mutation CAC526→TAC
MLRB331TGG TCTGGGCCCGGGTGGTTT Introduction of point mutation GCG33→CTG MLRB333CG TCTGCGCCGGGCCGGGTGGTTT Introduction of point mutation GCG33→CCG MLRB347ATC GGGTGCACGTCACGGATCTCTAGCC Introduction of point mutation GCG33→CCG MTRBWTF GCGAATTCTTGGCAGATCCCGGCGGGGG Cloning of M. tuberculosis rpoB, forward MTRBWTF GCGACGCTGGGCGCGGGGGGC Cloning of M. tuberculosis rpoB, forward MTRBWTF1 AATTCATGGACCAGAACAACCGCCT Introduction of point mutation at codons 506-508 MTRBWTF2 CTGTCGGCGCTGGGGCGCGGGGGTC Introduction of point mutation at codons 522, 523, 526, and 531 MTRBWTR2 CGGGCCTGGGTGGGTGGTGGTGAACCCCGGAGAA Introduction of point mutation at codons 512, 523, 526, and 511 MTRBS07AGC GGCTCAGCTGGCTGGTGGTGGTGAACCCGGAGAA Introduction of point mutation GGC507→AGC MTRB507AGC GGCTCAGCTGGGCTGGGCGGAAGAA Introduction of point mutation GC508→CGC MTRB507AGC GGCTCAGCTGGGTGGTGGCGAAGAA Introduction of point mutation GC507→AGC MTRB507AGT GGCTCAGCTGGGTGGCGCGAAGAA Introduction of point mutation GAC508→CGC MTRB507AGT GGCTCAGCTGGGTGGCGCGAGAAA Introduction of point mutation GAC507→AGC MTRB507AGT GGCTCAGCTGGGTGGTGGCGCGAGAAA Introduction of point mutation GAC507→AGC	MLRB531TTG	TGTTGGCGCTGGGCCCGGGTGGTTT	Introduction of point mutation TCG531→TTG
MIRB332TCG TGTCGTCGCCGGGGGGGTGTT Introduction of point mutation GCG332→CCG MIRB352TCG GGGTGCGGGCCGGGGTGGGTT Introduction of point mutation GTC333→CCG MIRBS47ATC GGGAGCTGCACGGACTCTCAGCC Introduction of point mutation GTC347→ATC MIRBWTF GCGAATTCTTGGCACAGAATCCTCGACAC Cloning of M. tuberculosis rpoB, forward MTRBWTF GCGAACTTTGGCACAGAACACCCGCC Cloning of M. tuberculosis rpoB, forward MTRBWTF1 AATTCATGGACCAGAACAACCCGGCT Introduction of point mutation at codons 505.508 MTRBWTF2 CTGTCGGCGGGGGCCCGGGGGCC Introduction of mutation at codons 514.515.518, 519, and 521. deletion MTRB9076AT GCGCTCAGCTGGGTGAACCCCGAC Introduction of point mutation GGC307→ACC MTRB5076AT GCCTCAGCTGGCTGGTGGCGCGAGAAA Introduction of point mutation GCC307→ACC MTRB507AG GCCTCAGCTGGCTGGTGGCGCGAAGAA Introduction of point mutation CGC307→ACC MTRB507AG GCCTCAGCTGGCTGGTGGCGCAAGAAA Introduction of point mutation CG531→TG MTRB507AG GCCTCAGCTGGCTGGTGGCGCAAGAA Introduction of point mutation CG531→ATC MTRB507AG GCCTCAGCTGGCTGGTGGCGCAAGAA Introduction of point mutation CAG511→CAC MTRB507AG GCCTCAGCTGGCTGGTGGCGCAAGAA Introduction of point mutation CAG511→CAC	MLRB531TGG	TGTGGGCGCTGGGCCCGGGTGGTTT	Introduction of point mutation TCG531→TGG
MLRB333CCG TGTCGCCCCGGGCCCCGGGTGTTT Introduction of point mutation GTG333—CCG MLRB333CCG GGTGCACCGTCACGGATCCCCAGCC Introduction of point mutation GTG347—ATC MLRB37ATC GCGAATTCCTGGCAGATCCCCGCCAGG Cloning of M. tubercalosis rpoB, forward MTRBWTF GCGAATTCTGGCCGAAACAACCGCCT Introduction of point mutation at codons 507, 508, 510, 511, 512, and MTRBWTF1 AATTCATGGACCGGAACAAACCGCGCT Introduction of point mutation at codons 524, 516, 518, 519, and 521; deletion MTRBWTF2 CTGTCGGCGCTGGTGGCGAAGAA Introduction of point mutation GCG37—AGC MTRBWTR1 GGCTCAGCTGGCTGGTGGTGCAACCCCGAC Introduction of point mutation GCG37—AGC MTRBS07CG GGCTCAGCTGGCTGGTGGCGAAGAA Introduction of point mutation GCG37—AGC MTRB507CG GGCTCAGCTGGCTGGTGGCGAAGAA Introduction of point mutation ACC508—GCC MTRB508CC GGCTCAGCTGGCTGGTGGCCGAAGAA Introduction of point mutation CAS13—CAC MTRB510CAT GGCTCAGCTGGCGCGAAGAA Introduction of point mutation CAS13—AAT MTRB513CG GGCTCAGCTGGCGCGAAGAA Introduction of point mutation CAS13—AAT MTRB513CAG GGCTCAGCTGGCGCGAAGAA Introduction of point mutation CAS13—AAT MTRB513CAA GGCTCAGCTGGCGCGCGAAGAA Introduction of point mutation CAS13—AAT	MLRB532TCG	TGTCGTCGCTGGGCCCGGGTGGTTT	Introduction of point mutation GCG532→TCG
MLRB94/ATC GGGTGCACGTCACGACATCTCTCACCC Introduction of point mutation GTC54/—ATC M. tuberculosis MTRBWTF GCGAAGTTCTTGGCAGATTCCCGCCAGAG Cloning of M. tuberculosis rpoB, forward MTRBWTF GCAAGCTTTACGCAAGATCCTCGACAC Cloning of M. tuberculosis rpoB, forward MTRBWTF1 AATTCATGGACCAGAACCCGCT Introduction of point mutation at codons 507, 508, 510, 511, 512, and MTRBWTF2 CTGTCGGCGGTGGTGGCGCGAGGGTC Introduction of point mutation at codons 504, 516, 518, 519, and 521; deletion MTRBWTR2 TCGGCGCTGGTGGTGGTGAGAGAA Introduction of point mutation GGC507—AGC MTRB507AGC GGCTCAGCTGGCTGGTGGTGAGAGAA Introduction of point mutation CGC508—CAC MTRB507AGC GGCTCAGCTGGCTGGTGGCGAAGAA Introduction of point mutation CGC508—CAC MTRB507AGC GGCTCAGCTGGCTGGTGGCCGAAGAA Introduction of point mutation CAS13—CCC MTRB507AGC GGCTCAGCTGGCTGGTGGCCGAAGAA Introduction of point mutation CAS13—AAT MTRB510CAT GGCTCAGCTGGCTGGTGGCCGAAGAA Introduction of point mutation CAS13—AAT MTRB513AAT1 GCTCAGCTGGCTGGTGCCCGAAGAA Introduction of point mutation CAS13—AAT MTRB513AAT2 ATTCATGGACCAGAAACAACCCGCT Introduction of point mutation CAS13—AAT MTRB513AAT1 GCTCAGCTGGGCTGGCCGAAGAA In	MLRB533CCG	TGTCGGCGCCGGGGCCCGGGTGGTTT	Introduction of point mutation CTG533→CCG
Mr. Rbevrelosis MTRBWTF GCAATCTTTGGCAGATTCCCGCCAGAG Cloning of M. tuberculosis rpoB, forward MTRBWTR GCAAGCTTTACGCAGATTCCCCGCAGCA Cloning of M. tuberculosis rpoB, forward MTRBWTR AATTCATGGACCAGAACAACCCGCT Introduction of point mutation at codons 507, 508, 510, 511, 512, and MTRBWTR1 GGCTCAGCTGGGGGCCGAGGGGCC Introduction of point mutation at codons 524, 516, 518, 519, and 521, deletion MTRBWTR2 TCGGCGCTTGTGGGTCAACCCCGAC Introduction of point mutation at codons 514, and 514, and 512, deletion MTRBS07AC GGCTCAGCTGGCTGGTGGTCGAAGAA Introduction of point mutation GGC507—AGC MTRBS08CAC GGCTCAGCTGGCTGGTGGCCGAAGAA Introduction of point mutation ACC508—CAC MTRBS08CCAG GGCTCAGCTGGCTGGGCGCGAAGAA Introduction of point mutation ACC508—CAC MTRBS10CAT GGCTCAGCTGGGCGGAGCAAAAA Introduction of point mutation CAG510—CAC MTRBS10AT1 TGCTCAGCTGGGTGGTGCCGAAGAA Introduction of point mutation CAG510—CAC MTRBS10AT2 GGCTCAGCTGGCGCGGCGCGAAGAA Introduction of point mutation CAS11—AAT MTRBS10AT1 TGCTCAGCTGGCTGGTGCCGAAGAA Introduction of point mutation CAS11—AAT MTRBS10AC GGCTCAGCTGGCGCGCGCGCGCCC Introduction of point mutation CAS16—GCC MTRBS10CAC	MLRB547ATC	GGGTGCACGTCACGGATCTCTAGCC	Introduction of point mutation GTC547→ATC
MTRBWTF GCGAAGTTCTTGGCAGATTCCCGCCAGAG Cloning of M. tuberculosis rpoB, forward MTRBWTR GCAAGCTTTACGGACAGATCCTCGACAC Cloning of M. tuberculosis rpoB, reverse MTRBWTF1 AATTCATGGACCAGAACACCCGCT Introduction of point mutation at codons 520, 528, 510, 511, 512, and 513 and deletion of codons 520, 523, 526, and 531 MTRBWTR1 GGCTCAGCTGGCTGGGCCGAGGAGAA Introduction of point mutation at codons 521, 526, and 531 MTRBWTR2 TCGGCGCTTGTGGGTCAACCCCGAC Introduction of point mutation at codons 522, 523, beta and 515 MTRBS07AGC GGCTCAGCTGGCTGGTCCGAAGAA Introduction of point mutation GCG37->AGC MTRBS08CAC GGCTCAGCTGGCTGGCGCGAAGAA Introduction of point mutation GCC30->AGC MTRBS08CAC GGCTCAGCTGGCTGGCGCGAAGAA Introduction of point mutation GCC30->AGC MTRBS10CAT GGCTCAGCTGGCTGGCGCGAAGAA Introduction of point mutation CAC508->GCC MTRBS10CAT GGCTCAGCTGGCTGGCTGCGAAGAA Introduction of point mutation CAG511->CCG MTRBS13AT1 TGCTCAGCTGGCTGGCGCGAAGAA Introduction of point mutation CAG51->AAT MTRBS13AT1 TGCTCAGCTGGCTGGCGCGAAGAA Introduction of point mutation CAG51->CG MTRBS13AT1 TGCTCAGCTGGCTGGCGCGAAGAA Introduction of point mutation CAC51->AAT	M. tuberculosis		
MTRBWTR GCAAGCTTTACGCAAGATCCTCGACAC Cloning of M. theoralosis rp81, reverse MTRBWTFI AATTCATGGACCAGAACACCCGCT Introduction of point mutation at codons 507, 508, 510, 511, 512, and 513 and deletion of codons 505, 508 MTRBWTR2 CTGTCGGCGCTGGGGGCCGGCGGTC Introduction of point mutation at codons 514, 516, 518, 519, and 521; deletion of codon 518; and insertion of TTC between codons 514 and 515 MTRBWTR2 TCGGCGCTGGTGGTGCTGAAGCAC Introduction of point mutation GCC507—AGCA MTRBS07AGC GGCTCAGCTGGCTGGTCCGAAGAA Introduction of point mutation GCC507—AGCA MTRBS07AGC GGCTCAGCTGGCTGGTCCGAAGAA Introduction of point mutation GCC508—CAC MTRBS07AGC GGCTCAGCTGGCTGGCCCGAAGAA Introduction of point mutation CAC508—CAC MTRBS08CC GGCTCAGCTGGCTGGCCCGAAGAA Introduction of point mutation CAC508—CAC MTRBS10CAT GGCTCAGCTGGCTGCCCGAAGAA Introduction of point mutation CAC510—CCAT MTRBS13AAT1 TGCTCGACGTGGTGCCCGAAGAA Introduction of point mutation CAS13—AAT MTRBS13AAT1 TGCTCGACGTGGTGCCCGAAGAA Introduction of point mutation CAS13—AAT MTRBS13AAT1 TGCTCGACGTGGTGCCCGAAGAA Introduction of point mutation CAS13—AAT MTRBS13AAT2 ATTCATGGACCAGAACAACCCGCT Introduction of point	MTRBWTF	GC <u>GAATTC</u> TTGGCAGATTCCCGCCAGAG	Cloning of M. tuberculosis rpoB, forward
MTRBWTF1AATTCATGGACCAGAACAACCCGCTIntroduction of point mutation at codons 507, 508, 510, 511, 512, and 513 and deletion of point mutation at codons 506, 508MTRBWTF2CTGTCGGCGCTGGGGCCCGAGGAGAIntroduction of point mutation at codons 514, 515, 518, 519, and 521 deletion of point mutation at codons 514, 516, 518, 519, and 521 deletion of point mutation at codons 514, 516, 518, 519, and 521 deletion of point mutation at codons 514, 516, 518, 519, and 521 deletion of codon 518, and insertion of TTC between codons 514 and 515MTRBVT2TCGGCGCTGGGGGGCGAAGCAIntroduction of point mutation GCS07-AGC GGCTCAGCTGGCTGGTGGCCGAAGAAMTRB507GATGGCTCAGCTGGCTGGTGGCCGAAGAAIntroduction of point mutation GCS07-AGCMTRB508GCCGGCTCAGCTGGCTGGTGCCGAAGAAIntroduction of point mutation CAS10CATMTRB510CATGGCTCAGCTGGCTGGTGCCGAAGAAIntroduction of point mutation CAS10CATMTRB510CATGGCTCAGCTGGTGGCGCGAAGAAIntroduction of point mutation CAS10CATMTRB513AAT1TGCTCAGCTGGCTGGTGCCGAAGAAIntroduction of point mutation CAS10AATMTRB513AAT2ATTCATGGACCAGAACAACCCGCTIntroduction of point mutation GAS16GAGMTRB513CACGCTCAGCTGGCTGGCGCAAGAAIntroduction of point mutation GAS16CACMTRB513CACATCATGGACCAGAACAACCCGCTIntroduction of point mutation GAS16CACMTRB513CACAATCCATGGACCAGAACAACCCCGCTIntroduction of point mutation GAS16CACMTRB513CACAATCATGGACCAGAACAACCCCGCTIntroduction of point mutation GAS16CACMTRB513CATATCATGGACCAGAACAACCCCGCTIntroduction of point mutation GAS16CACMTRB513CACCGGCGCTGTGGGCCAGACCCCCCCIn	MTRBWTR	GC <u>AAGCTT</u> TTACGCAAGATCCTCGACAC	Cloning of M. tuberculosis rpoB, reverse
513 and deletion of coloms 506-508 MTRBWTF2 CTGTCGGCGCTGGGGCCCGAGGGATC Introduction of point mutation at codons 512, 523, 526, and 531 MTRBWTR1 GGCTCAGCTGGTGGCGAAGAA Introduction of point mutation at codons 514, 516, 518, 519, and 521; deletion of codon 518; and insertion of TTC between codons 514 and 515 MTRB507AGC GGCTCAGCTGGCTGGTGGTGAAGAA Introduction of point mutation GGC507->AGC MTRB507CGT GGCTCAGCTGGCTGGTGGCCGAAGAA Introduction of point mutation ACC508->CCC MTRB508GCC GGCTCAGCTGGCTGGTGCCGAAGAA Introduction of point mutation ACC508->CCC MTRB511CCG GGCTCAGCTGGCTGGTGCCGAAGAA Introduction of point mutation CAG510->CAT MTRB513AT1 TGCTCAGCTGGCTGGTGCCGAAGAA Introduction of point mutation CAG510->CAT MTRB513AAT2 ATTCATGGACCAGACAACCCGGCT MTRB513GA CGCTCAGCTGGCGCGCGAAGAA MTRB516GC AATTCATGGACCAGACAACCCGGCT MTRB516GA CATCATGGCGCGAAACAACCCGGCT MTRB516GC AATTCATGGACCAGACAACCCGGCT MTRB516GC AATTCATGGACCAGAACAACCCGGCT MTRB521ATG AATTCATGGACCAGAACAACCCGGCT MTRB521ATG AATTCATGGACCAGACAACCCCGAC MTRB5221AT	MTRBWTF1	AATTCATGGACCAGAACAACCCGCT	Introduction of point mutation at codons 507, 508, 510, 511, 512, and
MTRBWTF2 CTGTCGGGGCTGGGGCCCGGCGTC Introduction of point mutation at codons 522, 523, 526, and 531 MTRBWTR1 GGCTCAGCTGGGTGGCGAGAGAA Introduction of point mutation at codons 514, 516, 518, 519, and 521; deletion of codon 518, and insertion of TTC between codons 514 and 515 MTRBS07AGC GGCTCAGCTGGCTGGTGGCGAAGAA Introduction of point mutation GGCS07→AGC MTRB507AGC GGCTCAGCTGGCTGGTGGCGAAGAA Introduction of point mutation GGCS07→AGC MTRB508CC GGCTCAGCTGGCTGGCCGAAGAA Introduction of point mutation ACC508→CAC MTRB508CC GGCTCAGCTGGCTGGTGCCGAAGAA Introduction of point mutation ACC508→CAC MTRB513CAT GGCTCAGCTGGCTGGTGCCGAAGAA Introduction of point mutation CAS510→CAT MTRB513AT1 TGCTCAGCTGGCTGGTGCCGAAGAA Introduction of point mutation CAS510→CAT MTRB513AT2 ATTCATGGACCAGAACAACCCGCT Introduction of point mutation CAS513→AAT MTRB516GA AATTCATGGACCAGAACAACCCGCT Introduction of point mutation GAS16→GAG MTRB5121ATG AATTCATGGACCAGAACAACCCCGCT Introduction of point mutation CAS516→GAG MTRB521ATG AATTCATGGACCAGAACAACCCCGCT Introduction of point mutation GAC516→GAG MTRB521ATG AATTCATGGACCAGAACAACCCCGCT Introduction of point mutation CAS216→ATG MTRB521ATG AATTCATGGACCAGAACAA			513 and deletion of codons 506-508
MTRBWTR1 GGCTCAGCTGGCTGGTGCCGAAGAA Introduction of mutation at codons 514, 516, 518, 519, and 521; deletion of codon 518; and insertion of TTC between codons 514 and 515 MTRBWTR2 TCGGCGCTTGTGGGTCAACCCCGAC Introduction of point mutation GCG507→AGC MTRB507GAT GGCTCAGCTGGCTGGTGGCCGAAGAA Introduction of point mutation GCG507→AGC MTRB508CAC GGCTCAGCTGGCTGGTGGCCGAAGAA Introduction of point mutation ACC508→CAC MTRB508CAC GGCTCAGCTGGCTGGTGCCGAAGAA Introduction of point mutation CAC508→CAC MTRB510CAT GGCTCAGCTGGCTGGCCGAAGAA Introduction of point mutation CAS10→CAT MTRB511CCG GCCTCAGCTGGCTGGTGCCGAAGAA Introduction of point mutation CAS11→CAT MTRB513AAT1 TGCTCAGCTGGCTGGTGCCGAAGAA Introduction of point mutation CAS11→CAT MTRB513GAA CGCTCAGCTGGTGCCGAAGAA Introduction of point mutation CAS13→AAT MTRB513GAA CGCTCAGCTGGTGCCGAAGAA Introduction of point mutation CAS13→AAT MTRB516GAG AATTCATGGACCAGAACAACCCGCT Introduction of point mutation GAS16→GGC MTRB516GTC AATTCATGGACCAGAACAACCCGGCT Introduction of point mutation GAS16→GGC MTRB526TAC CGGCGCTTGTGGGGTCAACCCGAC Introduction of point mutation GGS21→ATG MTRB526GC TCGGCGCTTGTGGGTCAACCCCGAC Introduction of	MTRBWTF2	CTGTCGGCGCTGGGGCCCGGCGGTC	Introduction of point mutation at codons 522, 523, 526, and 531
MTRBWTR2 TCGGCGCTTGTGGGGTCAACCCCGAC Introduction of point mutations TCG31→TTC and TCG31→TTG MTRB507AGC GCCTCAGCTGGCTGGTCTGAAGAA Introduction of point mutation GCC507→AGC MTRB508CAC GGCTCAGCTGGCTGGTGCCGAAGAA Introduction of point mutation GCC507→AGC MTRB508CC GGCTCAGCTGGCTGGCGCGAAGAA Introduction of point mutation ACC508→CAC MTRB510CAT GGCTCAGCTGGCTGGCCGAAGAA Introduction of point mutation ACC508→CAC MTRB511CAT GGCTCGCTGGCTGGCCGAAGAA Introduction of point mutation CAS110→CAT MTRB513AAT1 TGCTCAGCTGGCTGGCCGAAGAA Introduction of point mutation CAS110→CAT MTRB513AAT2 ATTTCATGGACCAGAACAACCCGCT Introduction of point mutation CAS113→AAT MTRB516GA AATTCATGGACCAGAACAACCCGCT Introduction of point mutation GAC516→GAG MTRB516AC AATTCATGGACCAGAACAACCCGCT Introduction of point mutation GAC516→GAG MTRB516AC AATTCATGGACCAGAACAACCCGCT Introduction of point mutation GGC52→TTG MTRB5221TG TCGGCGCTTGTGGGTCAACCCCGAC Introduction of point mutation CG522→TTG MTRB523CCC TCGGCGCTTGTGGGTCAACCCCGAC Introduction of point mutation CAC526→CTC MTRB523CCC TCGGCGCTTGTGGGTCAACCCCGAC Introduction of point mutation CAC526→TAC MTRB524CTC </td <td>MTRBWTR1</td> <td>GGCTCAGCTGGCTGGTGCCGAAGAA</td> <td>Introduction of mutation at codons 514, 516, 518, 519, and 521; deletion</td>	MTRBWTR1	GGCTCAGCTGGCTGGTGCCGAAGAA	Introduction of mutation at codons 514, 516, 518, 519, and 521; deletion
MTRBVTR2 TCGGCGCTTGTGGGTCAACCCCGAC Introduction of point mutations TCG531→TTC and TCG531→TTG MTRB507AGC GGCTCAGCTGGCTGGTATCGAAGAA Introduction of point mutation GGC507→AGAT MTRB508CAC GGCTCAGCTGGCTGGTGCCGAAGAA Introduction of point mutation ACC508→CAC MTRB510CAT GGCTCAGCTGGCTGGTGCCGAAGAA Introduction of point mutation ACC508→CAC MTRB510CAT GGCTCAGCTGGCTGGTGCCGAAGAA Introduction of point mutation CAG510→CAT MTRB511CG GGCTCAGCTGGCTGGTGCCGAAGAA Introduction of point mutation CA6510→CAT MTRB513AAT1 TGCTCAGCTGGCTGGTGCCGAAGAA Introduction of point mutation CAA513→AAT MTRB513AAT2 ATTTCATGGACCAGAACAACCCGCT Introduction of point mutation CAA513→AAT MTRB516GA AATTCATGGACCAGAACAACCCGCT Introduction of point mutation GAC516→GAG MTRB516GC AATTCATGGACCAGAACAACCCGCT Introduction of point mutation GAC516→GAG MTRB516GTC AATTCATGGACCAGAACAACCCGCT Introduction of point mutation GAC516→GAC MTRB520GC TCGGCGCTTGTGGGTCAACCCCGCT Introduction of point mutation GAC516→GAC MTRB520GC TCGGCGCTTGTGGGTCAACCCCGCC Introduction of point mutation CG520→GAC MTRB520GC TCGGCGCTTGTGGGTCAACCCCGAC Introduction of point mutation CG5230→GCG MT			of codon 518; and insertion of TTC between codons 514 and 515
MTRB507GATGGCTCAGCTGGCTGGTGGCGAAGAAIntroduction of point mutation GGC507->AGCMTRB508CACGGCTCAGCTGGCTGGGCGAAGAAIntroduction of point mutation ACC508->CACMTRB508CACGGCTCAGCTGGCTGGGCGCGAAGAAIntroduction of point mutation ACC508->GCCMTRB510CATGGCTCAGCTGGCTGGCGCGAAGAAIntroduction of point mutation CG510->CATMTRB511CCGGGCTCAGCTGGCTGGTGCCGAAGAAIntroduction of point mutation CA511->CCGMTRB513AAT1TGCTCAGCTGGCTGGTGCCGAAGAAIntroduction of point mutation CAA513->AATMTRB513AAT2ATTTCATGGACCAGAACAACCCGCTIntroduction of point mutation GAC516->GAGMTRB516GAGAATTCATGGACCAGAACAACCCGCTIntroduction of point mutation GAC516->GAGMTRB516GAGAATTCATGGACCAGAACAACCCGCTIntroduction of point mutation GAC516->CACMTRB516GACAATTCATGGACCAGAACAACCCGCTIntroduction of point mutation GAC516->CACMTRB516GACAATTCATGGACCAGAACAACCCGCTIntroduction of point mutation GAC516->CACMTRB521ATGAATTCATGGACCAGAACAACCCGCTIntroduction of point mutation GGC512->ATGMTRB522TTGTCGGCGCTTGTGGGTCAACCCCGACIntroduction of point mutation GGC522->TGMTRB523GCTCGGCGCTTGAGGTCAACCCCGACIntroduction of point mutation CAC526->TCCMTRB526TCTCGGCGCTTGAGGTCAACCCCGACIntroduction of point mutation CAC526->TCMTRB526ACTCGGCGCTTGAGGTCAACCCCGACIntroduction of point mutation CAC526->TCMTRB526ACTCGGCGCTTGAGGTCAACCCCGACIntroduction of point mutation CAC526->ACCMTRB526AACTCGGCGCTTGTGGGTCAACCCCGACIntroduction of point mutation CAC526->ACCMTRB526AAC	MTRBWTR2	TCGGCGCTTGTGGGTCAACCCCGAC	Introduction of point mutations TCG531 \rightarrow TTC and TCG531 \rightarrow TTG
MTR850/GA1GGCTCAGCTGGCTGGTGGCCGAAGAAIntroduction of point mutation GGCS0/~GA1MTR8508GCCGGCTCAGCTGGCTGGGCGCGAAGAAIntroduction of point mutation ACC508~CACMTR8510CATGGCTCAGCTGGCTGGTGCCGAAGAAIntroduction of point mutation CAG510~CATMTR8511CCGGGCTCGGCTGGTGCCGAAGAAIntroduction of point mutation CA511~CCGMTR8513AAT1TGCTCAGCTGGCTGGTGCCGAAGAAIntroduction of point mutation CA513~AATMTR8513AAT2ATTTCATGGACCAGCAGAACAACCCGCTIntroduction of point mutation CA513~AATMTR8516AACGCTCAGCTGGCTGGTGCCGAAGAAIntroduction of point mutation CA513~GAAMTR8516GAAATTCATGGACAGAACAACCCGCTIntroduction of point mutation GAC516~CACMTR8516GCAATTCATGGACCAGAACAACCCGCTIntroduction of point mutation GAC516~CACMTR8516GTCAATTCATGGACCAGAACAACCCGCTIntroduction of point mutation CG521~ATGMTR8520GCTCGGCGCTTGTGGGTCAACCCCGACIntroduction of point mutation CG522~TTGMTR8523GCGTCGGCGCTTGTGGGTCAACCCCGACIntroduction of point mutation CG523~GCGMTR8523GCCTCGGCGCTTGTGGGTCAACCCCGACIntroduction of point mutation CAC526~TCCMTR8526GTCTCGGCGCTTGTGGGTCAACCCCCGACIntroduction of point mutation CAC526~TCCMTR8526GAC	MTRB507AGC	GGCTCAGCTGGCTGGTGCTGAAGAA	Introduction of point mutation GGC507 \rightarrow AGC
MTRB508ACC GGCTCAGCTGGCTGGCGGAAGAA Introduction of point mutation ACC508→GCC MTRB508CC GGCTCAGCTGGCTGGGCGCGAAGAA Introduction of point mutation ACC508→GCC MTRB510CAT GGCTCAGCTGGCTGGCGCGAAGAA Introduction of point mutation ACC508→GCC MTRB511CG GGCTCAGCTGGCTGGTGCCGAAGAA Introduction of point mutation CAG510→CAT MTRB513AAT1 ATTCCATGGACCAGAACAACCCGCT Introduction of point mutation CAA513→AAT MTRB513GAA CGCTCAGCTGGCTGGTGCCGAAGAA Introduction of point mutation CAA513→AAT MTRB513GAA CGCTCAGCTGGCTGGTGCCGAAGAA Introduction of point mutation CAA513→AAT MTRB516GAG AATTCATGGACCAGAACACCCGGCT Introduction of point mutation GAC516→GAG MTRB516GAC AATTCATGGACCAGAACACCCGGCT Introduction of point mutation GAC516→GTC MTRB521ATG AATTCATGGACCAGAACACCCGGAT Introduction of point mutation GAC516→GTC MTRB523GC TCGGCGCTTGTGGGTCAACCCCGAC Introduction of point mutation GG522→TTG MTRB523GC TCGGCGCTTGTGGGTCAACCCCGAC Introduction of point mutation GG523→GGC MTRB523GCC TCGGCGCTTGAGGTCAACCCCGAC Introduction of point mutation CAC526→TCC MTRB526GAC TCGGCGCTTGTAGGTCAACCCCGAC Introduction of point mutation CAC526→TAC MTRB526GAC	MIKB50/GAI	GGUIUAGUIGGUIGGIAIUGAAGAA	Introduction of point mutation GGC50/→GA1
MTRB500CC GGCTCAGATGGCTGGTGCCGAAGAA Introduction of point mutation CAG510→CCT MTRB511CCG GGCTCGGCTGGTGGCGAAGAA Introduction of point mutation CAG510→CCT MTRB513AAT1 TGCTCAGCTGGCTGGTGCCGAAGAA Introduction of point mutation CAG510→CAT MTRB513AAT2 ATTTCATGGACCAGAACAACCCGCT Introduction of point mutation CAA513→AAT MTRB516GAG AATTCATGGACCAGAACAACCCGCT Introduction of point mutation GAC516→GAG MTRB516GAG AATTCATGGACCAGAACAACCCGCT Introduction of point mutation GAC516→GAG MTRB516GAG AATTCATGGACCAGAACAACCCGCT Introduction of point mutation GAC516→GAG MTRB516GTC AATTCATGGACCAGAACAACCCGGCT Introduction of point mutation GAC516→GAC MTRB521ATG AATTCATGGACCAGAACAACCCGGCT Introduction of point mutation GAC516→GAC MTRB523GGG TCGGCGCTTGTGGGTCAACCCCGAC Introduction of point mutation GGG523→GCG MTRB523GGC TCGGCGCTTGTGGGTCAACCCCGAC Introduction of point mutation CAC526→CTC MTRB526TAC TCGGCGCTTGAGGTCAACCCCGAC Introduction of point mutation CAC526→TAC MTRB526TAC TCGGCGCTTGAGGTCAACCCCGAC Introduction of point mutation CAC526→TCC MTRB526AC TCGGCGCTTGAGGTCAACCCCGAC Introduction of point mutation CAC526→ACA MTRB526AC	MTRD508CAC		Introduction of point mutation ACC508 CCC
MTRB511CCGGGCTCGGTGGCGGTGGCGCGAAGAAIntroduction of point mutation CAG510→CATMTRB513AAT1TGCTCAGCTGGCTGGTGCCGAAGAAIntroduction of point mutation CAG513→AATMTRB513AAT2ATTTCATGGACCAGAACAACCCGCTIntroduction of point mutation CAA513→AATMTRB513GAACGCTCAGCTGGCTGGTGCCGAAGAAIntroduction of point mutation CAA513→AATMTRB516GAGAATTCATGGACCAGAACAACCCGCTIntroduction of point mutation GAC516→GAGMTRB516GACAATTCATGGACCAGAACAACCCGCTIntroduction of point mutation GAC516→CACMTRB516GTCAATTCATGGACCAGAACAACCCGCTIntroduction of point mutation GAC516→GTCMTRB521ATGAATTCATGGACCAGAACAACCCGCATIntroduction of point mutation CG521→ATGMTRB523GCGTCGGCGCTTGTGGGTCAACCCCCAACIntroduction of point mutation GG523→GCGMTRB523GGCTCGGCGCTTGTGGGTCAACCCCGACIntroduction of point mutation CAC526→TTGMTRB526GACTCGGCGCTTGTAGGTCAACCCCGACIntroduction of point mutation CAC526→TACMTRB526TACTCGGCGCTTGTGGGTCAACCCCGACIntroduction of point mutation CAC526→TACMTRB526GACTCGGCGCTTGTGGGTCAACCCCGACIntroduction of point mutation CAC526→TACMTRB526GACTCGGCGCTTGTGGGTCAACCCCGACIntroduction of point mutation CAC526→ACMTRB526GACTCGGCGCTTGTGGGTCAACCCCGACIntroduction of point mutation CAC526→ACMTRB526AACTCGGCGCTTGTGGGGTCAACCCCGACIntroduction of point mutation CAC526→ACMTRB526AACTCGGCGCTTGTGGGTCAACCCCGACIntroduction of point mutation CAC526→ACMTRB526AACTCGGCGCTTGTGGGTCAACCCCGACIntroduction of point mutation CAC526→ACMTRB526AA <t< td=""><td>MTRB510CAT</td><td>GCTCAGCTGGCTGGCGCGAAGAA</td><td>Introduction of point mutation CAC510-CAT</td></t<>	MTRB510CAT	GCTCAGCTGGCTGGCGCGAAGAA	Introduction of point mutation CAC510-CAT
MTRB513AAT1GGCTCAGCTGGCTGGCGGAGAAIntroduction of point mutation CA513→AATMTRB513AAT2ATTTCATGGACCAGAACAACCCGCTIntroduction of point mutation CAA513→AATMTRB513GAACGCTCAGCTGGCTGGTGCCGAAGAAIntroduction of point mutation CAA513→AATMTRB516GAGAATTCATGGACCAGAACAACCCGCTIntroduction of point mutation GAC516→GAGMTRB516GACAATTCATGGACCAGAACAACCCGCTIntroduction of point mutation GAC516→CACMTRB516GTCAATTCATGGACCAGAACAACCCGCTIntroduction of point mutation GAC516→CACMTRB516GTCAATTCATGGACCAGAACAACCCGCTIntroduction of point mutation GAC516→CACMTRB521ATGAATTCATGGACCAGAACAACCCGATIntroduction of point mutation GG521→ATGMTRB523GGCTCGGCGCTTGTGGGTCAACCCCCAACIntroduction of point mutation GG523→GCGMTRB523GCGTCGGCGCTTGTGGGTCAACCCCGACIntroduction of point mutation CAC526→TCCMTRB526TACTCGGCGCTTGTAGGTCAACCCCGACIntroduction of point mutation CAC526→TACMTRB526GACTCGGCGCTTGTGGGTCAACCCCGACIntroduction of point mutation CAC526→TACMTRB526GACTCGGCGCTTGTGGGTCAACCCCGACIntroduction of point mutation CAC526→TACMTRB526GACTCGGCGCTTGTGGGTCAACCCCGACIntroduction of point mutation CAC526→TACMTRB526GACTCGGCGCTTGTGGGTCAACCCCGACIntroduction of point mutation CAC526→ACCMTRB526GACTCGGCGCTTGTGGGGTCAACCCCGACIntroduction of point mutation CAC526→CGCMTRB526AATCGGCGCTTGTGGGTCAACCCCGACIntroduction of point mutation CAC526→CGCMTRB526AATCGGCGCTTGTGGGTCAACCCCGACIntroduction of point mutation CAC526→CGCMTRB526AA	MTRB511CCC	GCTCCCCTCCCTCCCCAACAA	Introduction of point mutation CTG511 →CCG
MTRB513AAT2ATTTCATGGACCAGACACCCGCTIntroduction of point mutation CAA513 →AATMTRB513GAACGCTCAGCTGGCTGGTGCCGAAGAAIntroduction of point mutation CAA513→GAAMTRB516GAGAATTCATGGACCAGAACAACCCGCTIntroduction of point mutation GAC516→GAGMTRB516CACAATTCATGGACCAGAACAACCCGCTIntroduction of point mutation GAC516→CACMTRB516GTCAATTCATGGACCAGAACAACCCGCTIntroduction of point mutation GAC516→CACMTRB521ATGAATTCATGGACCAGAACAACCCGCTIntroduction of point mutation GAC516→CACMTRB522TTGTCGGCGCTTGTGGGTCAACCCCGACIntroduction of point mutation GG521→ATGMTRB523GCGTCGGCGCTTGTGGGTCAACCCCGACIntroduction of point mutation GG523→GGCMTRB526GACTCGGCGCTTGTGGGTCAACCCCGACIntroduction of point mutation GAC526→CTCMTRB526TACTCGGCGCTTGTAGGGTCAACCCCGACIntroduction of point mutation CAC526→CTCMTRB526TACTCGGCGCTTGTAGGTCAACCCCGACIntroduction of point mutation CAC526→TACMTRB526TACTCGGCGCTTGTAGGTCAACCCCGACIntroduction of point mutation CAC526→CTCMTRB526TACTCGGCGCTTGTAGGTCAACCCCGACIntroduction of point mutation CAC526→TACMTRB526TACTCGGCGCTTGTGGTCAACCCCGACIntroduction of point mutation CAC526→CTCMTRB526CACTCGGCGCTTGTGGGTCAACCCCGACIntroduction of point mutation CAC526→CACMTRB526CACTCGGCGCTTGTGGGTCAACCCCGACIntroduction of point mutation CAC526→CACMTRB526CAATCGGCGCTTGTGGGTCAACCCCGACIntroduction of point mutation CAC526→CACMTRB526CAATCGGCGCTTGTGGGTCAACCCCGACIntroduction of point mutation CAC526→CAAMTRB529AAA<	MTRB513AAT1	TGCTCAGCTGGCTGGTGCCGAAGAA	Introduction of point mutation CAA513 \rightarrow AAT
MTRB513GAACGCTCAGCTGGCTGGTGCCGAAGAAIntroduction of point mutation CAA513→GAAMTRB516GAGAATTCATGGAGCAGAACAACCCGCTIntroduction of point mutation GAC516→GAGMTRB516CACAATTCATGGCACCAGAACAACCCGGCTIntroduction of point mutation GAC516→CACMTRB516GTCAATTCATGGACCAGAACAACCCGGTIntroduction of point mutation GAC516→GAGMTRB521ATGAATTCATGGACCAGAACAACCCGGATIntroduction of point mutation CTG521→ATGMTRB523GCGTCGGCGCTTGTGGGTCAACCCCCAACIntroduction of point mutation GG523→GCGMTRB523GGCTCGGCGCTTGTGGGTCAACCCCGACIntroduction of point mutation GG523→GGCMTRB526TACTCGGCGCTTGTAGGGTCAACCCCGACIntroduction of point mutation CAC526→CTCMTRB526TACTCGGCGCTTGTAGGTCAACCCCGACIntroduction of point mutation CAC526→CTCMTRB526GACTCGGCGCTTGAGGTCAACCCCGACIntroduction of point mutation CAC526→CTCMTRB526GACTCGGCGCTTGAGGTCAACCCCGACIntroduction of point mutation CAC526→TTCMTRB526GACTCGGCGCTTGAGGTCAACCCCGACIntroduction of point mutation CAC526→TACMTRB526GACTCGGCGCTTGTGGGTCAACCCCGACIntroduction of point mutation CAC526→TCCMTRB526GACTCGGCGCTTGTGGGTCAACCCCGACIntroduction of point mutation CAC526→CACMTRB526CAATCGGCGCTTGTGGGTCAACCCCGACIntroduction of point mutation CAC526→CACMTRB520AATTGCGCCTGGGGGCCACCCGACIntroduction of point mutation CAC526→CACMTRB520AATTGCGCCTGGGGGCCAGCGGCCIntroduction of point mutation CAC520→CAAMTRB520AATTGCGCCTGTGGGGCCACCCGACIntroduction of point mutation CAC520→CAAMTRB531TTCCTGTT	MTRB513AAT2	ATTTCATGGACCAGAACAACCGCT	Introduction of point mutation CAA513 \rightarrow AAT
MTRB516GAGAATTCATGGAGCAGAACAACCCGCTIntroduction of point mutation GAC516→GAGMTRB516CACAATTCATGGCACCAGAACAACCCGCTIntroduction of point mutation GAC516→CACMTRB516GTCAATTCATGGTCCAGAACAACCCGCTIntroduction of point mutation GAC516→GTCMTRB521ATGAATTCATGGACCAGAACAACCCGATIntroduction of point mutation TCG521→ATGMTRB522TTGTCGGCGCTTGTGGGTCAACCCCAACIntroduction of point mutation TCG523→GCGMTRB523GCGTCGGCGCTTGTGGGTCAACCCCGACIntroduction of point mutation GGG523→GCGMTRB526GTCTCGGCGCTTGTGGGTCAACCCCGACIntroduction of point mutation GGG523→GCGMTRB526CTCTCGGCGCTTGTAGGGTCAACCCCGACIntroduction of point mutation CAC526→TCCMTRB526GTCTCGGCGCTTGTAGGTCAACCCCGACIntroduction of point mutation CAC526→TACMTRB526GACTCGGCGCTTGTAGGTCAACCCCGACIntroduction of point mutation CAC526→GACMTRB526GACTCGGCGCTTGTGGGTCAACCCCGACIntroduction of point mutation CAC526→GACMTRB526CTCTCGGCGCTTGTGGGTCAACCCCGACIntroduction of point mutation CAC526→AACMTRB526CACTCGGCGCTTGTGGGTCAACCCCGACIntroduction of point mutation CAC526→CACMTRB526CACTCGGCGCTTGTGGGTCAACCCCGACIntroduction of point mutation CAC526→CAAMTRB526CAATCGGCGCTTGTGGGGCCACCCGACCIntroduction of point mutation CAC526→CAAMTRB526CAATCGGCGCTTGTGGGGCCACCCGCACIntroduction of point mutation CAC526→CAAMTRB526AATTGCGCCTGGGGCCAACCCCGACIntroduction of point mutation CAC526→CAAMTRB526AATCGGCGCTTGTGGGGCCAGCCGCGCGCCIntroduction of point mutation CAC520→AAAMTRB526AA <td>MTRB513GAA</td> <td>CGCTCAGCTGGCTGGTGCCGAAGAA</td> <td>Introduction of point mutation CAA513—GAA</td>	MTRB513GAA	CGCTCAGCTGGCTGGTGCCGAAGAA	Introduction of point mutation CAA513—GAA
MTRB516CACAATTCATGCACCAGAACAACCCGCTIntroduction of point mutation GAC516 \rightarrow CACMTRB516GTCAATTCATGGTCCAGAACAACCCGATIntroduction of point mutation GAC516 \rightarrow GTCMTRB521ATGAATTCATGGACCAGAACAACCCGATIntroduction of point mutation CTG521 \rightarrow ATGMTRB522TTGTCGGCGCTTGTGGGTCAACCCCAACIntroduction of point mutation CG522 \rightarrow TTGMTRB523GGCTCGGCGCTTGTGGGTCAACCCCGACIntroduction of point mutation GGG523 \rightarrow GGCMTRB526CTCTCGGCGCTTGTGGGTCAACCCCGACIntroduction of point mutation CAC526 \rightarrow CTCMTRB526TACTCGGCGCTTGTAGGTCAACCCCGACIntroduction of point mutation CAC526 \rightarrow TACMTRB526GACTCGGCGCTTGTGGGTCAACCCCGACIntroduction of point mutation CAC526 \rightarrow TACMTRB526GACTCGGCGCTTGTAGGTCAACCCCGACIntroduction of point mutation CAC526 \rightarrow TACMTRB526AACTCGGCGCTTGTGGGTCAACCCCGACIntroduction of point mutation CAC526 \rightarrow CACMTRB526CATCGGCGCTTGTGGGTCAACCCCGACIntroduction of point mutation CAC526 \rightarrow CACMTRB529AAATTTGCGCGTTGTGGGTCAACCCCGACIntroduction of point mutation CAC526 \rightarrow CAAMTRB531TTCCTGTTCGCGCTGGGGCCCGGCGGTCIntroduction of point mutation TCG531 \rightarrow TTCMTRB531TTGCTGTTGGCGCTGGACCCCGGCGGTCIntroduction of point mutation TCG531 \rightarrow TTGMTRB506dGGCTCAGCTGGCTGAACCCCTGATIntroduction of point mutation 506-508del <td>MTRB516GAG</td> <td>AATTCATGGAGCAGAACAACCCGCT</td> <td>Introduction of point mutation GAC516\rightarrowGAG</td>	MTRB516GAG	AATTCATGGAGCAGAACAACCCGCT	Introduction of point mutation GAC516 \rightarrow GAG
MTRB516GTCAATTCATGGTCCAGAACAACCCGCTIntroduction of point mutation GAC516→GTCMTRB521ATGAATTCATGGACCAGAACAACCCGATIntroduction of point mutation CTG521→ATGMTRB522TTGTCGGCGCTTGTGGGTCAACCCCAACIntroduction of point mutation TCG522→TTGMTRB523GCGTCGGCGCTTGTGGGTCAACGCCGACIntroduction of point mutation GGG523→GCGMTRB523GCCTCGGCGCTTGTGGGTCAACCCCGACIntroduction of point mutation CAC526→CTCMTRB526CTCTCGGCGCTTGTAGGTCAACCCCGACIntroduction of point mutation CAC526→CTCMTRB526TACTCGGCGCTTGTGGGTCAACCCCGACIntroduction of point mutation CAC526→CTCMTRB526TACTCGGCGCTTGTGGGTCAACCCCGACIntroduction of point mutation CAC526→GACMTRB526TACTCGGCGCTTGTGGGTCAACCCCGACIntroduction of point mutation CAC526→GACMTRB526TACTCGGCGCTTGTGGGTCAACCCCGACIntroduction of point mutation CAC526→GACMTRB526CACTCGGCGCTTGTGGGTCAACCCCGACIntroduction of point mutation CAC526→AACMTRB526CACTCGGCGCTTGTGGGTCAACCCCGACIntroduction of point mutation CAC526→CACMTRB526CAATCGGCGCTTGTGGGTCAACCCCGACIntroduction of point mutation CAC526→CAAMTRB526CAATCGGCGCTTGTGGGTCAACCCCGACIntroduction of point mutation CAC526→CAAMTRB526CAATCGGCGCTTGTGGGTCAACCCCGACIntroduction of point mutation CAC526→CAAMTRB526CAATCGGCGCTTGGGGTCAACCCCGACIntroduction of point mutation CAC526→CAAMTRB526CAATCGGCGCTTGGGGCCAGGCGGTCIntroduction of point mutation CAC529→AAAMTRB531TTCCTGTTGGCGCTGGGGCCCGGCGGTCIntroduction of point mutation TCG531→TTCMTRB5306d <t< td=""><td>MTRB516CAC</td><td>AATTCATGCACCAGAACAACCCGCT</td><td>Introduction of point mutation GAC516\rightarrowCAC</td></t<>	MTRB516CAC	AATTCATGCACCAGAACAACCCGCT	Introduction of point mutation GAC516 \rightarrow CAC
MTRB521ATGAATTCATGGACCAGAACAACCCGATIntroduction of point mutation CTG521→ATGMTRB522TTGTCGGCGCTTGTGGGTCAACCCCCAACIntroduction of point mutation TCG522→TTGMTRB523GCGTCGGCGCTTGTGGGTCAACGCCGACIntroduction of point mutation GGG523→GCGMTRB523GGCTCGGCGCTTGTGGGTCAACCCCGACIntroduction of point mutation CAC526→CTCMTRB526CTCTCGGCGCTTGTAGGTCAACCCCGACIntroduction of point mutation CAC526→TACMTRB526TACTCGGCGCTTGTAGGTCAACCCCGACIntroduction of point mutation CAC526→ACCMTRB526TACTCGGCGCTTGTAGGTCAACCCCGACIntroduction of point mutation CAC526→ACCMTRB526ACTCGGCGCTTGTAAGGTCAACCCCGACIntroduction of point mutation CAC526→ACCMTRB526AACTCGGCGCTTGTGGTCAACCCCGACIntroduction of point mutation CAC526→ACCMTRB526AACTCGGCGCTTGTGGGTCAACCCCGACIntroduction of point mutation CAC526→ACCMTRB526CACTCGGCGCTTGTGGGTCAACCCCGACIntroduction of point mutation CAC526→AACMTRB526CAATCGGCGCTTGTGGGTCAACCCCGACIntroduction of point mutation CAC526→CGCMTRB529AAATTTGCGCTTGTGGGTCAACCCCGACIntroduction of point mutation CAC526→CAAMTRB531TTCCTGTTGGGGTCAACCIntroduction of point mutation CAC520→AAAMTRB531TTGCTGTTGGCGCTGGGGCCCGGCGGTCIntroduction of point mutation TCG531→TTCMTRB506dGGCTCAGCTGGCCCGGCGGTCIntroduction of point mutation TCG531→TTG	MTRB516GTC	AATTCATGGTCCAGAACAACCCGCT	Introduction of point mutation GAC516→GTC
MTRB522TTGTCGGCGCTTGTGGGTCAACCCCAACIntroduction of point mutation TCG522→TTGMTRB523GCGTCGGCGCTTGTGGGTCAACGCCGACIntroduction of point mutation GGG523→GCGMTRB523GGCTCGGCGCTTGTGGGTCAAGCCCGACIntroduction of point mutation CAC526→CTCMTRB526TCTCGGCGCTTGTAGGTCAACCCCGACIntroduction of point mutation CAC526→TACMTRB526GACTCGGCGCTTGTAGGTCAACCCCGACIntroduction of point mutation CAC526→ACCMTRB526ACTCGGCGCTTGTAGGTCAACCCCGACIntroduction of point mutation CAC526→ACCMTRB526AACTCGGCGCTTGTAGGTCAACCCCGACIntroduction of point mutation CAC526→ACCMTRB526AACTCGGCGCTTGTGGTCAACCCCGACIntroduction of point mutation CAC526→AACMTRB526CACTCGGCGCTTGTGGGTCAACCCCGACIntroduction of point mutation CAC526→AACMTRB526CACTCGGCGCTTGTGGGTCAACCCCGACIntroduction of point mutation CAC526→CGCMTRB526CAATCGGCGCTTGTGGGTCAACCCCGACIntroduction of point mutation CAC526→CGCMTRB529AAATTTGCGCTTGTGGGTCAACCCCGACIntroduction of point mutation CAC520→CGCMTRB531TTCCTGTTGGGGCCAGGGGCCCGGCGGTCIntroduction of point mutation TCG531→TTCMTRB531TTGCTGTTGGCGCTGGGGCCCGGCGGTCIntroduction of point mutation TCG531→TTGMTRB506dGGCTCAGCTGGCGCGACCCTGAACTCCTTGATIntroduction of mutation 506-508del	MTRB521ATG	AATTCATGGACCAGAACAACCCGAT	Introduction of point mutation CTG521→ATG
MTRB523GCGTCGGCGCTTGTGGGTCAACGCCGACIntroduction of point mutation GGG523→GCGMTRB523GGCTCGGCGCTTGTGGGTCAAGCCCGACIntroduction of point mutation GGG523→GGCMTRB526CTCTCGGCGCTTGTAGGGTCAACCCCGACIntroduction of point mutation CAC526→CTCMTRB526TACTCGGCGCTTGTAGGTCAACCCCGACIntroduction of point mutation CAC526→ACCMTRB526GACTCGGCGCTTGTAGGTCAACCCCGACIntroduction of point mutation CAC526→GACMTRB526TACTCGGCGCTTGTAAGGTCAACCCCGACIntroduction of point mutation CAC526→ACCMTRB526ACTCGGCGCTTGTGGTCAACCCCGACIntroduction of point mutation CAC526→AACMTRB526CACTCGGCGCTTGTGGGTCAACCCCGACIntroduction of point mutation CAC526→AACMTRB526CAATCGGCGCTTTGGGGTCAACCCCGACIntroduction of point mutation CAC526→CGCMTRB526CAATCGGCGCTTTGGGGTCAACCCCGACIntroduction of point mutation CAC526→CACMTRB529AAATTTGCGCTTGGGGTCAACCIntroduction of point mutation CAC520→CAAMTRB531TTCCTGTTGGCGCTGGGGCCCGGCGGTCIntroduction of point mutation TCG531→TTCMTRB530FAGGGCTCAGCTGGGGCCCGGCGGTCIntroduction of point mutation TCG531→TTGMTRB506dGGCTCAGCTGGCGCAGACTCCTTGATIntroduction of mutation 506-508del	MTRB522TTG	TCGGCGCTTGTGGGTCAACCCCAAC	Introduction of point mutation TCG522→TTG
MTRB523GGCTCGGCGCTTGTGGGTCAAGCCCGACIntroduction of point mutation GGG523→GGCMTRB526CTCTCGGCGCTTGAGGGTCAACCCCGACIntroduction of point mutation CAC526→CTCMTRB526TACTCGGCGCTTGTAGGTCAACCCCGACIntroduction of point mutation CAC526→GACMTRB526GACTCGGCGCTTGTAGGTCAACCCCGACIntroduction of point mutation CAC526→GACMTRB526AACTCGGCGCTTGTAGGTCAACCCCGACIntroduction of point mutation CAC526→ACCMTRB526AACTCGGCGCTTGTGGTCAACCCCGACIntroduction of point mutation CAC526→AACMTRB526CACTCGGCGCTTGTGGGTCAACCCCGACIntroduction of point mutation CAC526→AACMTRB526CAATCGGCGCTTTGGGTCAACCCCGACIntroduction of point mutation CAC526→CGCMTRB529AAATTTGCGCTTGTGGGTCAACCCCGACIntroduction of point mutation CAC520→CAAMTRB531TTCCTGTTGGGGTCAACCIntroduction of point mutation CAC520→AAAMTRB531TTGCTGTTGGCGCTGGGGCCCGGCGGTCIntroduction of point mutation TCG531→TTCMTRB506dGGCTCAGCTGGCTGAACTCCTTGATIntroduction of mutation 506-508del	MTRB523GCG	TCGGCGCTTGTGGGTCAACGCCGAC	Introduction of point mutation GGG523→GCG
MTRB526CTCTCGGCGCTTGAGGGTCAACCCCCGACIntroduction of point mutation CAC526 \rightarrow CTCMTRB526TACTCGGCGCTTGTAGGTCAACCCCCGACIntroduction of point mutation CAC526 \rightarrow TACMTRB526GACTCGGCGCTTGTCGGTCAACCCCCGACIntroduction of point mutation CAC526 \rightarrow GACMTRB526TTCTCGGCGCTTGTGGTCAACCCCCGACIntroduction of point mutation CAC526 \rightarrow AACMTRB526AACTCGGCGCTTGTGGGGTCAACCCCCGACIntroduction of point mutation CAC526 \rightarrow AACMTRB526CGCTCGGCGCTTGCGGGTCAACCCCCGACIntroduction of point mutation CAC526 \rightarrow CGCMTRB526CAATCGGCGCTTTTGGGTCAACCCCCGACIntroduction of point mutation CAC526 \rightarrow CAAMTRB529AAATTTGCGCTTGTGGGTCAACCIntroduction of point mutation CGA529 \rightarrow AAAMTRB531TTCCTGTTCGCGCTGGGGCCCGGCGGTCIntroduction of point mutation TCG531 \rightarrow TTCMTRB506dGGCTCAGCTGGCGCTGAACTCCTTGATIntroduction of mutation 506-508del	MTRB523GGC	TCGGCGCTTGTGGGTCAAGCCCGAC	Introduction of point mutation GGG523→GGC
MTRB526TACTCGGCGCTTGTAGGTCAACCCCGACIntroduction of point mutation CAC526 \rightarrow TACMTRB526GACTCGGCGCTTGTCGGTCAACCCCGACIntroduction of point mutation CAC526 \rightarrow GACMTRB526TTCTCGGCGCTTGAAGGTCAACCCCGACIntroduction of point mutation CAC526 \rightarrow AACMTRB526AACTCGGCGCTTGTGGGGCCAACCCCGACIntroduction of point mutation CAC526 \rightarrow AACMTRB526CGCTCGGCGCTTGCGGGTCAACCCCGACIntroduction of point mutation CAC526 \rightarrow CGCMTRB526CAATCGGCGCTTTTGGGTCAACCCCGACIntroduction of point mutation CAC526 \rightarrow CAAMTRB529AAATTTGCGCTTGTGGGTCAACCIntroduction of point mutation CAC529 \rightarrow AAAMTRB531TTCCTGTTCGCGCTGGGGCCCGGCGGTCIntroduction of point mutation TCG531 \rightarrow TTCMTRB506dGGCTCAGCTGGCGCGAACTCCTTGATIntroduction of mutation 506-508del	MTRB526CTC	TCGGCGCTTGAGGGTCAACCCCGAC	Introduction of point mutation CAC526→CTC
MTRB526GACTCGGCGCTTGTCGGTCAACCCCGACIntroduction of point mutation CAC526 \rightarrow GACMTRB526TTCTCGGCGCTTGAAGGTCAACCCCGACIntroduction of point mutation CAC526 \rightarrow AACMTRB526AACTCGGCGCTTGTGGGGCCAACCCCGACIntroduction of point mutation CAC526 \rightarrow AACMTRB526CGCTCGGCGCTTGCGGGTCAACCCCGACIntroduction of point mutation CAC526 \rightarrow CGCMTRB526CAATCGGCGCTTTTGGGTCAACCCCGACIntroduction of point mutation CAC526 \rightarrow CAAMTRB529AAATTTGCGCTTGTGGGTCAACCIntroduction of point mutation CAC520 \rightarrow AAAMTRB531TTCCTGTTCGCGCTGGGGCCCGGCGGTCIntroduction of point mutation TCG531 \rightarrow TTCMTRB531TTGCTGTTGGCGCTGGGGCCCGGCGGTCIntroduction of point mutation TCG531 \rightarrow TTGMTRB506dGGCTCAGCTGGCTGAACTCCTTGATIntroduction of mutation 506-508del	MTRB526TAC	TCGGCGCTTGTAGGTCAACCCCGAC	Introduction of point mutation CAC526→TAC
MTRB526TTCTCGGCGCTTGAAGGTCAACCCCCGACIntroduction of point mutation CAC526→TTCMTRB526AACTCGGCGCTTGTTGGTCAACCCCCGACIntroduction of point mutation CAC526→AACMTRB526CGCTCGGCGCTTGCGGGTCAACCCCCGACIntroduction of point mutation CAC526→CGCMTRB526CAATCGGCGCTTTTGGGTCAACCCCCGACIntroduction of point mutation CAC526→CAAMTRB529AAATTTGCGCTTGTGGGTCAACCCCCGACIntroduction of point mutation CAC526→CAAMTRB531TTCCTGTTCGCGCTGGGGCCCGGCGGTCIntroduction of point mutation TCG531→TTCMTRB531TTGCTGTTGGCGCTGGGGCCCGGCGGTCIntroduction of point mutation TCG531→TTGMTRB506dGGCTCAGCTGGCTGAACTCCTTGATIntroduction of mutation 506-508del	MTRB526GAC	TCGGCGCTTGTCGGTCAACCCCGAC	Introduction of point mutation CAC526–>GAC
MTRB526AACTCGGCGCTTGTTGGTCAACCCCGACIntroduction of point mutation CAC526→AACMTRB526CGCTCGGCGCTTGCGGGGTCAACCCCCGACIntroduction of point mutation CAC526→CGCMTRB526CAATCGGCGCTTTTGGGGTCAACCCCGACIntroduction of point mutation CAC526→CAAMTRB529AAATTTGCGCTTGTGGGGTCAACCIntroduction of point mutation CGA529→AAAMTRB531TTCCTGTTCGCGCTGGGGCCCGGCGGTCIntroduction of point mutation TCG531→TTCMTRB531TTGCTGTTGGCGCTGGGGCCCGGCGGTCIntroduction of point mutation TCG531→TTGMTRB506dGGCTCAGCTGGCTGAACTCCTTGATIntroduction of mutation 506-508del	MTRB526TTC	TCGGCGCTTGAAGGTCAACCCCGAC	Introduction of point mutation CAC526 \rightarrow TTC
MTRB526CGCTCGGCGCTTGCGGGTCAACCCCGACIntroduction of point mutation CAC526→CGCMTRB526CAATCGGCGCTTTTGGGTCAACCCCGACIntroduction of point mutation CAC526→CAAMTRB529AAATTTGCGCTTGTGGGGTCAACCIntroduction of point mutation CG529→AAAMTRB531TTCCTGTTCGCGCTGGGGCCCGGCGGTCIntroduction of point mutation TCG531→TTCMTRB531TTGCTGTTGGCGCTGGGGCCCGGCGGTCIntroduction of point mutation TCG531→TTGMTRB506dGGCTCAGCTGGCTGAACTCCTTGATIntroduction of mutation 506-508del	MTRB526AAC	TCGGCGCTTGTTGGTCAACCCCGAC	Introduction of point mutation CAC526 \rightarrow AAC
MTRB520CAA TCGGCGCTTTTGGGTCAACCCCGAC Introduction of point mutation CAC526→CAA MTRB529AAA TTTGCGCTTGTGGGTCAACC Introduction of point mutation CGA529→AAA MTRB531TTC CTGTTCGCGCTGGGGCCCGGCGGTC Introduction of point mutation TCG531→TTC MTRB531TTG CTGTTGGCGCTGGGGCCCGGCGGTC Introduction of point mutation TCG531→TTG MTRB506d GGCTCAGCTGGCGCGACCTCTTGAT Introduction of mutation 506-508del	MTRB526CGC		Introduction of point mutation CAC526 \rightarrow CGC
MTRB522AAA TTGCGCTGTGGGGTCAACC Introduction of point mutation CGA229→AAA MTRB531TTC CTGTTCGCGCTGGGGGCCCGGCGGTC Introduction of point mutation TCG531→TTC MTRB531TTG CTGTTGGCGCTGGGGCCCGGCGGTC Introduction of point mutation TCG531→TTG MTRB506d GGCTCAGCTGGCTGAACTCCTTGAT Introduction of mutation 506-508del	MTDP520LAA	TTTCCCCTTCTCCCTCAACCUUGAU	Introduction of point mutation $CA(520 \rightarrow CAA)$
MTRB55117C CTGTTGGCGCTGGGGCCCCGGCGGTC Introduction of point initiation TCG551→TC MTRB506d GGCTCAGCTGGCTGAACTCCTTGAT Introduction of point mutation 506-508del	MTRR521TTC		Introduction of point mutation $TCC531 \rightarrow TTC$
MTRB506d GGCTCAGCTGGACTCCTTGAT Introduction of point initiation 1CG551->11G	MTRR531TTC	CTGTTGGCGCTGGCGCCCCCCCCCCCCCCCCCCCCCCCC	Introduction of point mutation TCG531-TTC
	MTRB506d	GGCTCAGCTGGCTGAACTCCTTGAT	Introduction of putation 506-508del
MTRBin514TTC AATTCTTCATGGACCAGAACAACCC Introduction of mutation 514insTTC	MTRBin514TTC	AATTCTTCATGGACCAGAACAACCC	Introduction of mutation 514insTTC
MTRBd518 AATTCATGGACCAGAACCCGCTGTC Introduction of mutation 518del	MTRBd518	AATTCATGGACCAGAACCCGCTGTC	Introduction of mutation 518del

^{*a*} Restriction sites are underlined.

TABLE 2 Rifampin and	d rifabutin susce	eptibilities of th	ne recombinant A	A. smegmatis strains
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	Rifampin	Rifampin		Rifabutin	
Mutation	MIC (µg/ml)	Fold increase ^a	MIC (µg/ml)	Fold increase	Reference(s)
M. leprae					
Wild type	1		0.25		
$GGC507 \rightarrow GGG \text{ (silent)}$	1	1	0.25	1	This study
GGC507→AGC (G507S)	0.5	0.5	0.125	0.5	3
$ACC508 \rightarrow ACA (silent)$	1	1	0.25	1	This study
CAG513→GTG (O513V)	32	32	8	32	3
GAT516→AAT (D516N)	32	32	2	8	14
CAG517→CAT (O517H)	1	1	0.25	1	11
CAC526→TAC (H526Y)	32	32	8	32	14
TCG531→TTG (S531L)	32	32	4	16	3, 14
TCG531→TGG (S531W)	32	32	8	32	14
GCG532→TCG (A532S)	1	1	0.25	1	11
CTG533→CCG (L533P)	32	32	4	16	14
GTC547→ATC (V547I)	1	1	0.25	1	This study
M. tuberculosis					
Wild type	1		0.25		
GGC507→AGC (G507S)	0.5	0.5	0.125	0.5	1
GGC507→GAT (G507D)	0.5	0.5	0.125	0.5	1
ACC508→CAC (T508H)	0.5	0.5	0.125	0.5	1
ACC508→GCC (T508A)	1	1	0.25	1	1
CAG510→CAT (Q510H)	1	1	0.25	1	22
CTG511→CCG (L511P)	16	16	1	4	1, 12
CAA513→AAT (Q513N)	8	8	0.5	2	1
CAA513→GAA (Q513E)	32	32	2	8	1
GAC516→GAG (D516E)	8	8	0.5	2	12
GAC516→CAC (D516H)	1	1	0.25	1	1
GAC516→GTC (D516V)	32	32	2	8	12, 21, 22
CTG521→ATG (L521M)	1	1	0.125	0.5	21
TCG522→TTG (S522L)	>32	>32	8	32	21
GGG523→GCG (G523A)	1	1	0.125	0.5	1
GGG523→GGC (silent)	1	1	0.25	1	1
CAC526→CTC (H526L)	32	32	4	16	12, 22
CAC526→TAC (H526Y)	>32	>32	8	32	12, 22
CAC526→GAC (H526D)	>32	>32	8	32	12, 22
CAC526→TTC (H526F)	>32	>32	4	16	1
CAC526→AAC (H526N)	32	32	2	8	8
CAC526→CGC (H526R)	32	32	8	32	12, 22
CAC526→CAA (H526Q)	8	8	0.5	2	1
CGA529→AAA (R529K)	32	32	4	16	22
TCG531→TTC (S531F)	32	32	4	16	1
TCG531→TTG (S531L)	32	32	8	32	21, 22
506-508del ^b	16	16	0.5	2	5
514insTTC ^c	>32	>32	8	32	12 22
518del ^d	32	32	2	8	22

^{*a*} Fold increase in MIC compared to the wild-type sequence.

^b Deletion of codons 506 to 508.

^{*c*} Insertion of TTC between codons 514 and 515.

^d Deletion of codon 518.

ic-exchange mutants were constructed by using a temperature-sensitive mycobacteriophage method described in a previous report (2). Using the *M. smegmatis* mc²155 genome sequence (GenBank accession number CP000480), the upstream and downstream flanking DNA sequences were used to generate a deletion mutation in the *rpoB* gene (MSMEG_1367). To disrupt the *rpoB* gene, DNA segments from 1,119 bp upstream through 21 bp downstream of the initiation codon of *M. smegmatis rpoB* and from 39 bp upstream through 941 bp downstream of the termination codon were cloned directionally into the cosmid vector pYUB854, which contains a *res-hyg-res* cassette and a *cos* sequence for lambda phage assembly.

The plasmids thus produced were digested with PacI and ligated into PH101 genomic DNA excised from the phage-plasmid hybrid (phasmid) phAE87 by PacI digestion. The ligated DNA was packaged (GigaPackIII Gold packaging extract; Stratagene, La Jolla, CA). The resultant mixture was used for the transduction of *E. coli* STBL2 cells (Life Technologies Inc., Carlsbad, CA) to yield cosmid DNA. After *E. coli* was transduced and the transductants were plated onto hygromycin-containing medium, phasmid DNA was prepared from the pooled antibiotic-resistant transductants and electroporated into *M. smegmatis* mc²155. Bacterial cells were incubated at 30°C to produce the recombinant phage. The *M. smeg*-



FIG 1 Construction of recombinant M. smegmatis strains for rifampin susceptibility testing.

matis transformant carrying the *M. leprae* or *M. tuberculosis rpoB* gene was infected with the produced temperature-sensitive phage at 37°C for allelic exchange, and kanamycin-resistant and hygromycin-resistant colonies were isolated. Two colonies for each point mutation were subjected to subsequent tests.

Drug susceptibility testing. The MIC values for *M. smegmatis* recombinant clones were determined by culture on Middlebrook 7H10 agar plates containing 2-fold serial dilutions of rifampin (0.25 to $32 \ \mu$ g/ml) or rifabutin (0.0625 to $8 \ \mu$ g/ml). The MIC value for each strain was defined as the lowest concentration of the drug necessary to inhibit bacterial growth.

RESULTS

Construction of recombinant *M. smegmatis* strains. In our previous study, we sequenced the *rpoB* regions of *M. leprae* clinical samples isolated in Vietnam and detected several mutations (11). In addition to these mutations, we detected some mutations (GGC \rightarrow GGG at codon 507, ACC \rightarrow ACA at codon 508, and GTC \rightarrow ATC at codon 547) in clinical specimens from Vietnam and other countries (our unpublished data). We prepared plasmids with mutations in the *M. leprae* and *M. tuberculosis rpoB* genes. Each plasmid has one of 40 mutations (12 for *M. leprae rpoB* and 28 for *M. tuberculosis rpoB*) presented in Table 2. The mutated sequences were confirmed by sequencing. Plasmids carrying the *M. leprae* or *M. tuberculosis rpoB* gene with or without a point mutation were introduced individually into *M. smegmatis*. The *M. smegmatis* transformants were subjected to allelic exchange to dis-

rupt the *rpoB* gene on their own chromosome (Fig. 1). The isolation of *rpoB*-disrupted mutants carrying the pNN301-*rpoB* constructs was unsuccessful. Consequently, the recombinant strains with pMV261-*rpoB* constructs were used for subsequent tests. PCR analysis confirmed that the *M. smegmatis rpoB* sequences in the recombinant strains with pMV261-*rpoB* constructs were replaced by hygromycin resistance gene sequences (see Fig. S1 in the supplemental material). All strains showed growth rates comparable to that of wild-type *M. smegmatis*.

Drug susceptibility. The rifampin susceptibilities and rifabutin susceptibilities of the recombinant M. smegmatis strains were tested (see Fig. S2 in the supplemental material). The MIC values of rifampin and rifabutin for the recombinant M. smegmatis strains and the fold increases in MIC compared to the wild-type sequences are presented in Table 2. It should be noted that the MIC values for the M. smegmatis strains might be shifted from those for M. leprae or M. tuberculosis because of their differences in cell wall permeability and other factors. The MIC value of rifampin for the recombinant *M. smegmatis* strain with the wild-type sequence of the M. leprae rpoB or M. tuberculosis rpoB gene was 1 μ g/ml. Most strains that had a mutation at codon 511, 513, 516, 522, 526, 531, or 533 showed rifampin resistance. In contrast, strains that had a mutation at codon 507, 508, 517, 521, 523, or 532 showed MIC values of rifampin comparable to those for the wildtype sequence. The MIC values of rifabutin for the recombinant



FIG 2 Mutations introduced into the *M. leprae rpoB* gene or *M. tuberculosis rpoB* gene and rifampin susceptibility. The consensus amino acid sequence of *M. leprae* RpoB and *M. tuberculosis* RpoB between codons 506 and 565 is shown. The *M. leprae rpoB* sequence and codons are shown above the consensus amino acid sequence. The *M. tuberculosis rpoB* sequence and codons are shown below the consensus sequence. Mutated codons that gave rise to rifampin resistance are surrounded by ovals. Mutated codons that showed levels of rifampin susceptibility comparable to those of the wild-type sequences are surrounded by rectangles.

M. smegmatis strains with the wild-type sequence of the *M. leprae* rpoB or *M. tuberculosis* rpoB gene were 0.25 μ g/ml. Generally, rifabutin was more efficacious than rifampin in terms of concentration.

DISCUSSION

To functionally replace the *rpoB* gene of *M. smegmatis* with the *M. leprae* or *M tuberculosis* counterpart, we used a method established in our previous study (16). Because *rpoB* is a necessary gene for bacterial growth, this genetic locus cannot be disrupted without compensating for its activity. Therefore, we first introduced the *rpoB* gene of *M. leprae* or *M. tuberculosis* into *M. smegmatis* using vector plasmids of two types before disrupting the *rpoB* gene on the *M. smegmatis* chromosome. One vector was pMV261, a multicopy shuttle plasmid. The other was a single-copy integrative shuttle plasmid, pNN301. However, the isolation of *rpoB*-disrupted mutants carrying pNN301-*rpoB* constructs was unsuccessful, probably because of insufficient RpoB expression.

We tested 2 silent mutations and 10 mutations that change amino acid residues for *M. leprae* (Fig. 2). Codons 516, 526, 531, and 533 in the *M. leprae rpoB* gene are known to be codons responsible for rifampin resistance. However, it remains unclear whether or not mutations that have not been reported previously can confer rifampin resistance. Our results show that not all mutations in the *rpoB* gene detected in *M. leprae* clinical samples confer rifampin resistance. *M. leprae* is not cultivable. Therefore, it has been very difficult to analyze the mutation-susceptibility relationship. Using recombinant *M. smegmatis*, however, we can analyze it in a few weeks. We also tested 1 silent mutation, 24 mutations that change amino acids, 2 deletions, and 1 insertion for *M. tuberculosis*. Some mutations did not confer rifampin resistance, which is inconsistent with the susceptibility of the *M. tuberculosis* clinical isolates reported previously. Most mutations at codon 516, 526, or 531 showed rifampin resistance. It is interesting that the strains with the mutation GAC516 \rightarrow CAC for D516H were not rifampin resistant. All other mutations at codon 516 showed rifampin resistance. The mutation GAC516 \rightarrow CAC in *M. tuberculosis* was reported for a strain with multiple mutations and should not be involved in rifampin resistance.

Rifabutin, a spiropiperidyl rifampin, is a rifamycin derivative that is more active than rifampin against slow-growing mycobacteria, including *M. tuberculosis* and *M. avium-M. intracellulare* complex strains, *in vitro* and *in vivo*. It is also active against some rifampin-resistant strains of *M. tuberculosis* (6, 13). Our results indicate that some mutations (e.g., GAT516 \rightarrow AAT of *M. leprae* and GAC516 \rightarrow GAG of *M. tuberculosis*) show weak resistance to rifabutin.

Molecular methods designed to detect drug resistance have some limitations. In some cases, the identified mutations are not related to the acquisition of resistance. Caution is necessary when considering mutations, especially if the mutation detected in clinical isolates is not reported very often. For example, Q510H and L521M mutations were detected in rifampin-resistant M. tuberculosis isolates (21, 22), but our results suggest that these mutations are not responsible for rifampin resistance (Table 2). The method used for this study can directly assess the influence of designated mutations in rpoB. If the mutations can confer rifampin resistance, we can eliminate the possibility that genetic variation in some region other than *rpoB* on the chromosome of the clinical isolates is responsible for the resistance. Bahrmand et al. previously reported the high-level rifampin resistance of M. tuberculosis isolates with multiple mutations within the rpoB gene (1). Our method might also be useful for analyzing multiple mutations

detected in the *rpoB* gene of clinical isolates to determine the contribution of each single mutation to rifampin resistance.

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