Supplementary Materials

Table S1: Sequences of primers and probes used in the study

Name	5'-end	Sequence (5'to3')	3'-end	Target mutation Site
RIF1-F3	N/A	AGCGGATGACCACCCAG	N/A	Primer Set-1
RIF1-B3	N/A	CGCTCACGTGACAGACCG	N/A	
RIF1-FIP	N/A	CTTGATCGCGGCGACCACCGGACGTGGAGGCGATCACA	N/A	
RIF1-BIP	N/A	GTTCTTCGGCACCAGCCAGCTGCCGACAGTCGGCGCTT	N/A	
RIF1-LF	N/A	GATGTTGATCAACGTCTGCG	N/A	
RIF1-LB	N/A	GAGCCAATTCATGGACCAGAA	N/A	
RIF2-F3	N/A	AGCGGATGACCACCCAG	N/A	Primer Set-2
RIF2-B3	N/A	TGCACGTCGCGGACCT	N/A	
RIF2-FIP	N/A	CTTGATCGCGGCGACCACCG GACGTGGAGGCGATCACA	N/A	
RIF2-BIP	N/A	CAGAACAACCCGCTGTCGG CACGCTCACGTGACAGACC	N/A	
RIF2-LF	N/A	GCCGGATGTTGATCAACG	N/A	
RIF2-LB	N/A	CCACAAGCGCCGACTG	N/A	
Probe C	HEX	CGCGAGCCGGATGTTGATCAACGTCGCG	BHQ-1	Calibrator Probe
Probe I_1	Cy5	CGCGACCAATTCATG[+G][+A][+C]CAGAACAACTCGCG	BHQ-2	Probe for mutation at 516
Probe I_2	FAM	CGCGAGACC[+C][+A][+C]AAGCGCCGACTG[+T][+C][+G]GCGCTCGCG	BHQ-1	Probe for mutation at 526

*: [+] is LNA modified base.

Table S2: 81-bp Rifampicin Resistance-Determining Region (RRDR) Sequences

Sample	Sequence type	81-bp RRDR sequences	Codon
WT	Wild Type	GGCACCAGCCAGCTGAGCCAATTCATGGACCAGAACAACCCGCTGTCGGGGT TGACCCACAAGCGCCGACTGTCGGCGCTG	Asp-516, His-526, Ser-531
Mut 1	Mutant	GGCACCAGCCAGCTGAGCCAATTCATGTACCAGAACAACCCGCTGTCGGGGT TGACCCACAAGCGCCGACTGTCGGCGCTG	**Tyr-516**, His-526, Ser-531
Mut 2	Mutant	GGCACCAGCCAGCTGAGCCAATTCATGGTCCAGAACAACCCGCTGTCGGGGT TGACCCACAAGCGCCGACTGTCGGCGCTG	**Val-516**, His-526, Ser-531
Mut 3	Mutant	GGCACCAGCCAGCTGAGCCAATTCATGGACCAGAACAACCCGCTGTCGGGGT TGACCTACAAGCGCCGACTGTCGGCGCTG	Asp-516, **Tyr-526**, Ser-531
Mut 4	Mutant	GGCACCAGCCAGCTGAGCCAATTCATGGACCAGAACAACCCGCTGTCGGGGT TGACCCTCAAGCGCCGACTGTCGGCGCTG	Asp-516, **Leu-526**, Ser-531
Mut 5	Mutant	GGCACCAGCCAGCTGAGCCAATTCATGGTCCAGAACAACCCGCTGTCGGGGT TGACCCTCAAGCGCCGACTGTTGGCGCTG	**Val-516**, **Leu-526**, Leu-531
Mut 6	Mutant	GGCACCAGCCAGCTGAGCCAATTCATGTACCAGAACAACCCGCTGTCGGGGT TGACCCTCAAGCGCCGACTGTGGGCGCTG	**Tyr-516**, **Leu-526**, Trp-531

Table S3: Reference training cohort data set

Reference	f_{ib1}	f_{ie1}	f_{ib2}	f_{ie2}	f_{cb}	f_{ce}	Probe I_1 $S_{ref}=(f_{ie1} / f_{ib1}) / (f_{ce} / f_{cb})$	Probe I_2 $S_{ref}=(f_{ie2} / f_{ib2}) / (f_{ce} / f_{cb})$
MTB-gDNA	3060	3684	3637	6016	2327	2679	1.05	1.44
MTB-gDNA	3082	3705	3637	5733	2311	2668	1.04	1.36
MTB-gDNA	3064	3676	3591	5471	2307	2654	1.04	1.32
MTB-gDNA	3033	3615	3516	5599	2284	2604	1.05	1.40
MTB-gDNA	3126	3570	3674	6154	2296	2657	0.99	1.45
MTB-gDNA	3077	3678	3552	5062	2282	2635	1.04	1.23

*Note: For Probe I_1: Mean (μ) S_{ref}=1.03, Standard Deviation (σ) S_{ref}=0.02
For Probe I_2: Mean (μ) S_{ref} =1.37, Standard Deviation (σ) S_{ref} =0.08

Table S4: Test cohort data set

Plasmid Type	f_{ib1}	f_{ie1}	f_{ib2}	f_{ie2}	f_{cb}	f_{ce}	Probe I_1		Probe I_2	
							$Ss=(f_{ie1} / f_{ib1}) / (f_{ce} / f_{cb})$	Z-score	$Ss=(f_{ie2} / f_{ib2}) / (f_{ce} / f_{cb})$	Z-score
WT	3091	3737	3707	5031	2326	2694	1.04	0.50	1.17	2.46
WT	3103	3708	3894	5593	2368	2750	1.03	0.18	1.24	1.64
WT	3088	3714	3723	5876	2329	2706	1.03	0.09	1.36	0.11
WT	3001	3552	3492	5201	2281	2630	1.03	0.28	1.29	0.95
WT	3116	3736	3650	5299	2297	2658	1.04	0.16	1.26	1.41
WT	3040	3650	3504	4783	2261	2620	1.04	0.15	1.18	2.38
Mut 1	3080	3432	3756	5594	2292	2677	0.95	3.43	1.28	1.16
Mut 1	3054	3412	3618	5453	2267	2637	0.96	3.18	1.30	0.90
Mut 1	3080	3452	3631	5715	2261	2642	0.96	3.22	1.35	0.25
Mut 1	3080	3458	3666	5217	2268	2673	0.95	3.52	1.21	2.02
Mut 1	3060	3421	3663	5488	2253	2633	0.96	3.34	1.28	1.07
Mut 1	3071	3405	3591	5552	2244	2599	0.96	3.30	1.33	0.41
Mut 2	3035	3358	3744	5005	2281	2666	0.95	3.77	1.14	2.81
Mut 2	3092	3445	3760	6351	2290	2687	0.95	3.65	1.44	0.90
Mut 2	3101	3473	3769	6379	2282	2676	0.96	3.41	1.44	0.96
Mut 2	3085	3444	3744	6523	2255	2631	0.96	3.33	1.49	1.58
Mut 2	3111	3465	3798	5810	2266	2649	0.95	3.51	1.31	0.74
Mut 2	3072	3448	3656	4746	2263	2666	0.95	3.52	1.10	3.34
Mut 3	3088	3719	3890	4656	2339	2718	1.04	0.16	1.03	4.24
Mut 3	3074	3683	3876	4685	2315	2739	1.01	0.90	1.02	4.35
Mut 3	3049	3626	3631	4526	2294	2658	1.03	0.28	1.08	3.67
Mut 3	3073	3660	3685	4599	2295	2712	1.01	1.10	1.06	3.92
Mut 3	3101	3749	3648	4295	2285	2676	1.03	0.03	1.01	4.56
Mut 3	3044	3632	3536	4391	2259	2628	1.03	0.32	1.07	3.78
Mut 4	3074	3667	3838	4785	2307	2697	1.02	0.54	1.07	3.79
Mut 4	3029	3570	3674	4492	2277	2650	1.01	0.87	1.05	3.99
Mut 4	3068	3656	3674	4706	2288	2679	1.02	0.66	1.09	3.44
Mut 4	3003	3498	3510	4398	2249	2604	1.01	1.17	1.08	3.59
Mut 4	3118	3693	3678	4455	2291	2681	1.01	0.90	1.04	4.18
Mut 4	3036	3600	3554	4451	2259	2628	1.02	0.61	1.08	3.67
Mut 5	3127	3521	3926	4814	2339	2820	0.93	4.34	1.02	4.41
Mut 5	3108	3502	3799	4638	2294	2748	0.94	4.04	1.02	4.38
Mut 5	3132	3530	3828	4724	2295	2751	0.94	4.06	1.03	4.26
Mut 5	3100	3494	3745	4557	2267	2712	0.94	3.97	1.02	4.41
Mut 5	3119	3507	3742	4590	2266	2706	0.94	4.00	1.03	4.28
Mut 5	3124	3514	3710	4528	2274	2701	0.95	3.76	1.03	4.27
Mut 6	3122	3545	3796	4798	2305	2768	0.95	3.83	1.05	3.96
Mut 6	3078	3469	3675	4629	2274	2721	0.94	3.97	1.05	3.96
Mut 6	3093	3495	3707	4681	2287	2730	0.95	3.77	1.06	3.90
Mut 6	3143	3563	3736	4804	2285	2729	0.95	3.64	1.08	3.65
Mut 6	3159	3573	3819	4781	2290	2745	0.94	3.92	1.04	4.06
Mut 6	3106	3482	3660	4600	2263	2685	0.94	3.86	1.06	3.88