Mutation-enriched Sanger sequencing. The PCR components for *katG* gene were as follows: 25μL PCR reaction contained 1 × PCR buffer [75 mM Tris-HCl (pH 9.0), 20 mM $(NH_4)_2SO_4$, and 0.01% (V/V) Tween 20], 4.0 mM $MgCl_2$, 0.2 mM dNTPs, 0.06 μM katG-F2, 1.2 μM katG-R, 0.16 μM katG-P, 1 U KlenTaq-S DNA polymerase, and 5 μL template. The PCR components for *inhA* promoter were as follows: 25μL PCR reaction contained 1 × PCR buffer [75 mM Tris-HCl (pH 9.0), 20 mM $(NH_4)_2SO_4$, and 0.01% (V/V) Tween 20], 4.0 mM $MgCl_2$, 0.2 mM dNTPs, 0.06 μM inhA-F, 0.6 μM inhA-R, 0.16 μM inhA-P, 1 U KlenTaq-S DNA polymerase, and 5 μL template. The PCR components for *ahpC* promoter were as follows: 25μL PCR reaction contained 1 × PCR buffer [75 mM Tris-HCl (pH 9.0), 20 mM $(NH_4)_2SO_4$, and 0.01% (V/V) Tween 20], 4.0 mM $MgCl_2$, 0.2 mM dNTPs, 0.08 μM ahpC-F, 1.0 μM ahpC-R, 0.16 μM ahpC-P1 and 0.16 μM ahpC-P2, 1 U KlenTaq-S DNA polymerase, and 5 μL template. The PCR conditions were identical: step 1, denaturation at 95 ℃ for 3 min, step 2, 95 ℃ for 10 s, 61 ℃ for 15 s, and 68 ℃ for 30 s, repeated 50 cycles. PCR products were sent out for bidirectional sequencing using the forward and reverse primers for each gene (Sangon Inc., Shanghai, China).

Table S1 Sequences of primers and probes used in the DeepMelt TB/INH assay

Name[a]	Target	Sequence (5'-3')[c]
katG-F1[b]	*katG*	CGTCGGCGGTCACACTTTCGGTAAGA
katG-F2[b]		CACCGGAACCGGTAAGGACGCG
katG-R		TCGTCAGCTCCCACTCGTAGCCGTA
inhA-F	*inhA* promoter	CGTTACGCTCGTGGACATACCGATTT
inhA-R		GGGATACGAATGGGGGTTTGGCCCCTTCA
ahpC-F	*ahpC* promoter	GCCACGGCCGGCTAGCACCTCTTG
ahpC-R		GCATGACTCTCCTCATCATCAAAGC
katG-P	*katG* 315	FAM-TCGATCACCAGCGGCATCGAG-BHQ1
inhA-P	*inhA* -17~-8	ROX-GCGGCGAGACGATAGGTTGTC-BHQ2
ahpC-P1	*ahpC* -44~-30	HEX-ATATATCACCTTTGCCTGACAGC-BHQ1
ahpC-P2	*ahpC* -15~4	BHQ2-ACGGCACGATGGAATGTCGCA-Cy5

[a] F, forward primer; R, reverse primer; P, probe.

[b] katG-F1 and katG-F2 were used in the singleplex and multiplex assay, respectively.

[c] Underlined bases in the probe sequences represent locked nucleic acid (LNA) modification.