Supplementary Figure legend:

Figure S1: **(A)** Three probe melting temperature profile of (i) *M. malmoensae* and (ii) *M. tuberculosis* with wild type RRDR DNA. Fifty nanograms of *M. malmoensae* DNA was used for the assay. **(B)** Melting temperature profiles of the three SMB probes for the wild type and the 516TAC mutant DNA in presence and absence of an excess of human genomic DNA added to the assay. Graph (i) *M. tuberculosis* DNA with wild type RRDR (ii) *M. tuberculosis* DNA with wild type RRDR in presence of human genomic DNA (iii) *M. tuberculosis* DNA with 516TAC mutation (iv) *M. tuberculosis* DNA with 516TAC mutation in presence of human genomic DNA.

Supplementary table 1: Three probe Tm code of NTM DNA mixed with *M. tuberculosis* DNA containing wild type and different mutant RRDR sequences

Sample	Tm rpo1	Tm rpo2	Tm rpo3
M. tuberculosis H37Rv	69.0	68.9	64.0
M. abscessus + Wild Type RRDR	68.7	68.9	63.9
M. abscessus + 516AAC	69.0	66.1	63.4
M. abscessus + 526CGC	68.8	69.0	59.4
M. abscessus + 531TTG	69.1	69.3	68.1
M. abscessus + 533CCG	68.9	69.2	65.4
M. gordonae + Wild Type RRDR	68.8	68.8	64.0
M. gordonae + 516AAC	69.2	65.9	63.5
M. gordonae + 526CGC	68.7	68.9	59.2
M. gordonae + 531TTG	69.2	69.4	68.1
M. gordonae + 533CCG	68.8	69.0	65.3
M. chelonae + Wild Type RRDR	69.0	69.2	64.1
M. chelonae + 516AAC	68.9	66.1	63.4
M. chelonae + 526CGC	68.9	69.2	59.4
M. chelonae + 531TTG	69.1	69.3	68.1
M. chelonae + 533CCG	68.9	68.9	65.3
M. avium + Wild Type RRDR	69.1	69.0	64.0
M.avium + 516AAC	68.9	66.0	63.4
M. avium + 526CGC	68.9	69.0	59.5
M. avium + 531TTG	69.0	69.3	68.0
M. avium + 533CCG	69.0	69.1	65.4
M. kansasii + Wild Type RRDR	68.9	69.1	64.1
M. kansasii + 516AAC	68.8	66.0	63.4
M. kansasii + 526CGC	68.8	69.1	59.4
M. kansasii + 531TTG	69.1	69.6	68.0
M. kansasii + 533CCG	68.8	69.2	65.3
M. intracellulare + Wild Type RRDR	68.9	69.2	64.6
M. intracellulare + 516AAC	68.9	65.7	63.2
M. intracellulare + 526CGC	68.9	69.0	59.4
M. intracellulare + 531TTG	69.2	69.3	68.1
M. intracellulare + 533CCG	68.9	69.1	65.3
M. malmoense + Wild Type RRDR	69.0	69.1	64.0
M. malmoense + 516AAC	68.9	70.2 - 65.4	63.7
M. malmoense + 526CGC	68.9	69.0	59.5
M. malmoense + 531TTG	69.2	69.4	68.3
M. malmoense + 533CCG	68.9	69.1	65.4
M. smegmatis + Wild Type RRDR	69.0	69.1	64.5
M. smegmatis + 516AAC	68.7	70.1 - 65.1	63.9
M. smegmatis + 526CGC	69.2	69.4	59.1
M. smegmatis + 531TTG	68.9	69.6	68.7
M. smegmatis + 533CCG	69.1	69.1	66.1