Supplement

Table S1. Summary of beacon performance per operator by visual analysis

Operator	Performance	Beacon sensitivity and specificity (%)					
		A	B	C	D	E	Overall
1	Sensitivity	100	93	100	85	5	59
	Specificity	100	100	100	100	100	100
2	Sensitivity	100	93	50	90	0	57
	Specificity	100	99	100	100	100	98
3	Sensitivity	0	87	50	85	14	63
	Specificity	100	98	100	100	100	98
4	Sensitivity	100	100	100	90	67	93
	Specificity	80	68	81	74	78	52

Light shade, beacon with poorest performance for each operator

Table S2. Distribution of mutations and corresponding amino acid changes within the 81-bp region of *rpoB* in drug-resistant isolates from Colombia and the Texas-Mexico border

Beacon	Affected codon[a]	Codon change[b]	Amino acid change[b]	Colombia (n=29)[c]	Texas-Mexico border (n=22)[c]
A	428	AGC → A**G**G	Ser → Arg	1 (3%)	0 (0%)
	429	CAG → CA**C**	Gln → His	0 (0%)	1 (5%)
	430	CTG → **A**TG	Leu → Met	1 (3%)	0 (0%)
B	433	TTC → TT**T**	Phe → Phe[d]	0 (0%)	1 (5%)
	435	GAC → **T**AC	Asp → Tyr	1 (3%)	1 (5%)
	435	GAC → G**T**C	Asp → Val	5 (14%)	2 (10%)
	435	GAC → G**G**C	Asp → Gly	0 (0%)	1 (5%)
	435	GAC → GA**G**	Asp → Glu	0 (0%)	1 (5%)
	435-436	**ΔGAC CAG**	ΔAspGln	0 (0%)	1 (5%)
C	441	TCG → **A**CG	Ser → Thr	2 (6%)	0 (0%)
	441	TCG → T**TC**	Ser → Phe	2 (6%)	0 (0%)
D	445	CAC → **T**AC	His → Tyr	2 (6%)	1 (5%)
	445	CAC → **A**AC	His → Asn	1 (3%)	1 (5%)
	445	CAC → **G**AC	His → Asp	2 (6%)	6 (29%)
	445	CAC → C**G**C	His → Arg	0 (0%)	4 (19%)
	447	CGC → CG**T**	Arg → Arg[d]	2 (6%)	0 (0%)
E	450	TCG → T**T**G	Ser → Leu	18 (49%)	1 (5%)
	450	TCG → T**G**G	Ser → Trp	1 (3%)	0 (0%)
	450	TCG → T**AC**	Ser → Tyr	0 (0%)	1 (5%)
	450	TCG → **C**GC	Ser → Arg	0 (0%)	1 (5%)

[a] Codon number based on *M. tuberculosis rpoB* (accession no. AE000516, nucleotides 761762-765298) with changed nucleotide shown in underlined bold; codon 447 was not covered by the beacons used in this study (shaded); [b] arrows indicate change from wild-type to mutant; ; [c] data expressed as n(column %); [d] silent mutations

Table S3. Quantification potential of beacon qPCR using known proportions of DNA from wild-type and mutant bacteria for beacon B[a]

DNA mix wildtype + mutant for beacon B	Expected		No. targets detected in beacon B	Observed	
	Wild-type (%)	Mutant (%)		Wild-type (%)	Mutant (%)
1000 + 0	100	0	726	100	0
700 + 300	70	30	538	74	26
500 + 500	50	50	312	43	57
300 + 700	30	70	253	35	65
100 + 900	10	90	45	6	94
1 + 999	1	99	0	0	100
0 + 1000	0	100	0	0	100

[a] Different ratios of DNA from a strain with no detectable Ct for beacon B (assumed 100% mutations) and DNA from another strain with a Ct suggesting no mutations in 100% of the bacteria were mixed in different proportions in the laboratory to evaluate the quantification potential of beacon qPCR. A total of 103 genomes were added per qPCR reaction for these experimental specimens, in parallel with a standard curve for beacon B which contained known amounts of wild-type DNA target as reference. The number of targets detected by beacon B is shown (# targets detected in beacon B), and percentage of mutants versus wild-type was estimated using as a reference the number of targets detected for beacon B when no mutations were added (1000 wild-type genomes, 726 targets).

	Beacon A		Beacon B		Beacon C		Beacon D		Beacon E		Beacon 16S rRNA	
A	S1	S1	S1	S1	S1	S1	S1	S1	S1	S1	S1	S1
B	S2	S2	S2	S2	S2	S2	S2	S2	S2	S2	S2	S2
C	S3	S3	S3	S3	S3	S3	S3	S3	S3	S3	S3	S3
D	S4	S4	S4	S4	S4	S4	S4	S4	S4	S4	S4	S4
E	S5	S5	S5	S5	S5	S5	S5	S5	S5	S5	S5	S5
F	S6	S6	S6	S6	S6	S6	S6	S6	S6	S6	S6	S6
G	CDC1551	CDC1551	CDC1551	CDC1551	CDC1551	CDC1551	CDC1551	CDC1551	CDC1551	CDC1551	CDC1551	CDC1551
H	NTC	NTC	NTC	NTC	NTC	NTC	NTC	NTC	NTC	NTC	NTC	NTC
	1	2	3	4	5	6	7	8	9	10	11	12

Fig S1. 96-well plate design. Every plate was loaded with six experimental strains (S1-S6; rows A thru F), a wild-type reference strain (CDC1551; row G), and a non-template control (NTC; row H). Beacons A – E for *rpoB* (columns 1 thru 10) and 16S rRNA for *M. tuberculosis* species confirmation (columns 11-12) were run in duplicates for each strain.

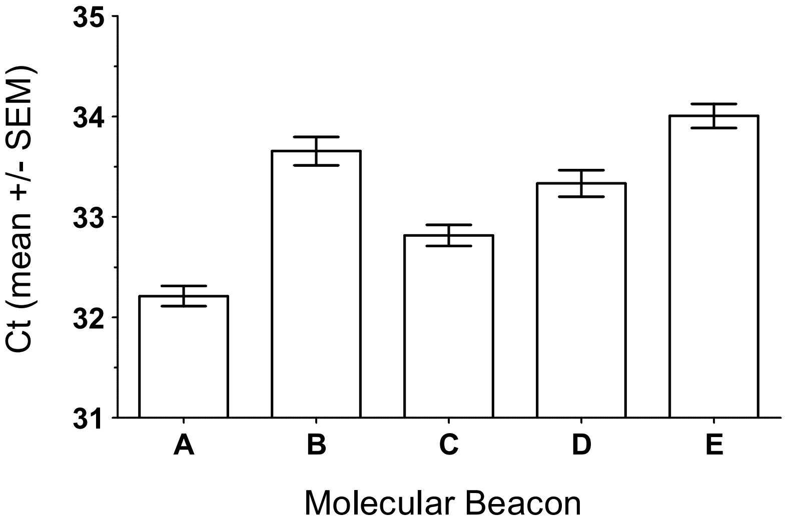

Fig S2. Efficiency, distribution and reproducibility of beacons A-E for strain CDC1551 for the 18 96-well plates ran in this study. Beacon A had a consistently lower raw Ct (mean±SEM; 32.2±0.10), followed by C (32.8±0.10), D (33.3±0.13), B (33.7±0.14) and E (34.0±0.12).