

# Simplified Model to Survey Tuberculosis Transmission in Countries Without Systematic Molecular Epidemiology Programs

## Appendix

**Appendix Table 1.** Specific SNP alleles targeted and PCR design for each *Mycobacterium tuberculosis* strain

Cluster	SNP per cluster	Position	SNP	Gen	Primer sequence (5' to 3')	Pool concentration, $\mu$ M	Target strain	PCR fragment size, bp
Cluster A, Beijing	SNP1	204386	G>A	<i>Rv0173</i>	Fw-Rv0173: GCTACCGAAAAATGCCACCG Rv-Rv0173: GAGCACCCGATTGAGGACT	2 2	Non-A strain	400
	SNP2	518748	G>A	<i>Rv0430</i>	Fw-Rv0430: CGATATGGACAGCGCCATGA Rv-Rv0430: TCACCTCGAAGCCAAGGC	6 6	Strain A	308
	SNP3	3479530	G>A	<i>Rv3111</i>	Fw-Rv3111: TGGAAATGGAAGCGTTGACC Rv-Rv3111: ACGAACGGCACCGAAGTAA	5 5	Non-A strain	228
Cluster B, Haarlem	SNP1	261991	G>A	<i>Rv0218</i>	Fw-Rv0218: CACCTGGAGCGGCGTA Rv-Rv0218: GCGTTGCAATGTCGAACATT	2 2	Strain B	297
	SNP2	825060	C>T	<i>Rv0732</i>	Fw-Rv0732: TGCCCTACATCACCGCC Rv-Rv0732: GATGTCCAGCGAGCAACC	2 2	Non-B strain	209
	SNP3	2425513	G>T	<i>RV2163c</i>	Fw-RV2163: CATCGAGCACGGGTTGAG Rv-RV2163: GGCCTTGATAATGCGTGGG	2 2	Non-B strain	406
	SNP4	568395	G>T	<i>Rv0479c</i>	Fw-Rv0479: GCATCCTTCGGTGTGGC Rv-Rv0479: GGATCGTGAGCTTTATCTGCATT	2 2	Strain B	127
Cluster C, LAM	SNP1	516278	G>T	<i>Rv0427c</i>	Fw-Rv0427: CGCACGTTTCCGAACCC Rv-Rv0427: CGATCTGGGCGTCCATCA	2 2	Non-C strain	207
	SNP2	781859	T>C	<i>Rv0682</i>	Fw-Rv0682: ACAACCTGCAGGAGCACTC Rv-Rv0682: CCCTGCGTATCCAGCGAA	6 6	Non-C strain	103
	SNP3	1802486	G>T	<i>Rv1601</i>	Fw-Rv1601: ACGCCAGCTTCGACCTC Rv-Rv1601: AACGATTGGAACACGTGCA	2 2	Strain C	307
	SNP4	4403893	T>C	<i>Rv3915</i>	Fw-Rv3915: AAGGACGGATGGCAGCC Rv-Rv3915: GAGAATGCCTTCGGCGATG	6 6	Strain C	413

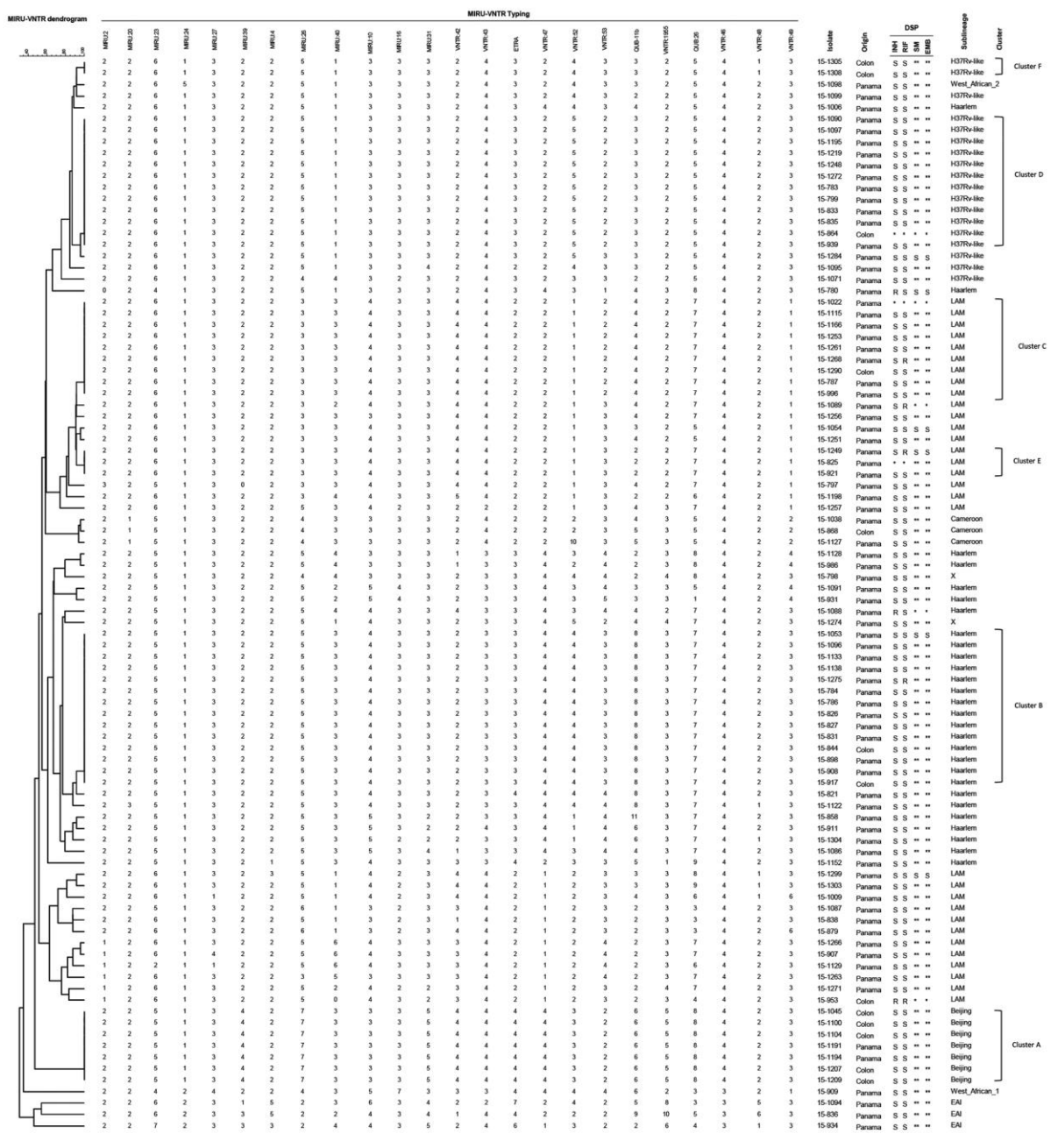
\*LAM, Latin American-Mediterranean; SNP, single-nucleotide polymorphism.

**Appendix Table 2.** Reaction mixes for each PCR\*

Reagent	Volume per reaction, $\mu\text{L}$		
	Cluster A	Cluster B	Cluster C
HotStarTaq polymerase, 5 U/ $\mu\text{L}$ diluted 1:10	1.25	1.25	1.25
MgCl <sub>2</sub> , 25 mM	0.4	0.3	0.4
PCR buffer, 10 $\times$ with 15 mM MgCl <sub>2</sub>	2.5	2.5	2.5
Q solution, 5 $\times$	5.0	5.0	5.0
Primer pool, 10 $\times$ †	2.5	2.5	2.5
dNTPs, 25 mM	0.2	0.2	0.2
H <sub>2</sub> O	10.65	10.75	10.7
DNA target		2.5 $\mu\text{L}$ for each one	
Final reaction volume of 25 $\mu\text{L}$			

\*dNTPs, deoxynucleotides; MgCl<sub>2</sub>, magnesium chloride.

†Primer pool concentration described in Appendix Table 1. The PCR conditions for each strain was as follows: 95° C for 15 min, followed by 27 cycles of 95° C for 1 min, annealing (6° C for Custer A and 60° C both for Custer B and C for 1 min) and 72° C for 10 min.



**Appendix Figure.** Mycobacterial interspersed repetitive unit–variable-number tandem-repeat data from a 2015 pilot study in Panama of a simplified model to survey the transmission of tuberculosis in countries without systematic molecular epidemiology programs. Genotypes were compared by using the unweighted pair group method of arithmetic averaging in Bionumerics version 4.61 (<http://www.applied-maths.com/bionumerics>). DSP, drug-susceptibility pattern; EMB, ethambutol; INH, isoniazid; LAM, Latin American–Mediterranean; R, resistant; RIF, rifampin; S, susceptible; SM, streptomycin; VNTR,<sub>i</sub> \*, no data; †, none evaluated.