

## Marker status for the competitions between engineered strains

Competition assays occurred for 6 isolates (2 from each treatment). For each isolate, all combinations of mutations in *rpoB* were constructed and then competitions were run between any pair of engineered strains differing by a single mutation at each of the concentrations of rifampicin listed in Supplementary Table 2. The following table details the marker status of each strain in each competition:

Ara markers in various env.'s		0		0.0001		0.0306		0.4189		2.3485		8.5116		23.7976		56.0084		116.593		190.000			
		+	-	+	-	+	-	+	-	+	-	+	-	+	-	+	-	+	-	+	-		
competitions																							
G13	ab vs. Ab	Ab	ab	Ab	ab	Ab	ab	Ab	ab	ab	Ab	ab	Ab	ab	ab	Ab	ab	Ab	ab	ab	Ab		
	ab vs. aB	aB	ab	aB	ab	aB	ab	aB	ab	ab	aB	ab	aB	ab	ab	aB	ab	aB	ab	ab	aB		
	Ab vs. AB	Ab	AB	Ab	AB	Ab	AB	Ab	AB	Ab	AB	Ab	AB	Ab	AB	Ab	AB	Ab	AB	Ab	AB		
	aB vs. AB	AB	aB	AB	aB	AB	aB	AB	aB	AB	aB	AB	aB	AB	aB	AB	aB	AB	aB	AB	aB	AB	
G18	ab vs. Ab	Ab	ab	Ab	ab	Ab	ab	Ab	ab	ab	Ab	ab	ab	Ab	ab	ab	Ab	ab	ab	Ab	ab		
	ab vs. aB	aB	ab	aB	ab	aB	ab	aB	ab	ab	aB	ab	ab	aB	ab	ab	aB	ab	ab	aB	ab		
	Ab vs. AB	Ab	AB	Ab	AB	Ab	AB	Ab	AB	Ab	AB	Ab	AB	Ab	AB	Ab	AB	Ab	AB	Ab	AB		
	aB vs. AB	AB	aB	AB	aB	AB	aB	AB	aB	AB	aB	AB	aB	AB	aB	AB	aB	AB	aB	AB	aB	AB	
M358	ab vs. Ab	Ab	ab	Ab	ab	Ab	ab	Ab	ab	ab	Ab	ab	ab	Ab	ab	ab	Ab	ab	ab	Ab	ab		
	ab vs. aB	aB	ab	aB	ab	aB	ab	aB	ab	ab	aB	ab	ab	aB	ab	ab	aB	ab	ab	aB	ab		
	Ab vs. AB	Ab	AB	Ab	AB	Ab	AB	Ab	AB	Ab	AB	Ab	AB	Ab	AB	Ab	AB	Ab	AB	Ab	AB		
	aB vs. AB	AB	aB	AB	aB	AB	aB	AB	aB	AB	aB	AB	aB	AB	aB	AB	aB	AB	aB	AB	aB	AB	
M367	ab vs. Ab	Ab	ab	Ab	ab	Ab	ab	Ab	ab	ab	Ab	ab	ab	Ab	ab	ab	Ab	ab	ab	Ab	ab		
	ab vs. aB	aB	ab	aB	ab	aB	ab	aB	ab	ab	aB	ab	ab	aB	ab	ab	aB	ab	ab	aB	ab		
	Ab vs. AB	AB	Ab	AB	Ab	AB	Ab	AB	Ab	AB	Ab	AB	Ab	AB	Ab	AB	Ab	AB	Ab	AB	Ab	AB	
	aB vs. AB	aB	AB	aB	AB	aB	AB	aB	AB	aB	AB	aB	AB	aB	AB	aB	AB	aB	AB	aB	AB	aB	AB
S 497	a vs. A	a	A	a	A	A	A	a	A	a	A	a	A	a	A	a	A	a	A	a	A		
	a vs. A	a	A	a	A	A	A	a	A	a	A	a	A	a	A	a	A	a	A	a	A		
S1236	a vs. A	a	A	a	A	A	A	a	A	a	A	a	A	a	A	a	A	a	A	a	A		
	a vs. A	a	A	a	A	A	A	a	A	a	A	a	A	a	A	a	A	a	A	a	A		

**Table 1.** Each column represents a different concentration of rifampicin (from 0 µg/mL to 190 µg/mL). Each row signifies a pair of competing engineered genotypes (four replicates were done for each competition). Each column is broken down into two sub-columns, labeled “+” and “-”, representing each competing strain marked Ara+ and Ara-, respectively. The strain marked Ara+ was expected to lose in competition (based on growth rate measured in the spectrophotometer). This was done so that we could track this strain at low relative density (by plating on minimal arabinose). The genotype abbreviations are as follows: for *Gradual* population 13 (G13), ab (bases g428 and c1721), Ab (bases a428 and c1721), aB (bases g428 and t1721) and AB (bases a428 and t1721); for *Gradual* population 18 (G18), ab (bases a443 and c1527), Ab (bases t443 and c1527), aB (bases a443 and a1527) and AB (bases t443 and a1527); for *Moderate* population 358 (M358), ab (bases t437 and a1685), Ab (bases a437 and a1685), aB (bases t437 and c1685) and AB (bases a437 and c1685); for *Moderate* population 367 (M367), ab (bases t1532 and g1546), Ab (bases g1532 and g1546), aB (bases t1532 and a1546) and AB (bases g1532 and a1546); for *Sudden* population 497 (S497), a (base g1546), and A (base t1546); and for *Sudden* population 1236 (S1236), a (base g436), and A (base t436).