

Neutrality of marker used for the competition assay

In order to assess whether the Ara marker was neutral, we followed the basic competition protocol (see Methods). We tracked several competitions, each between a strain marked Ara+ and the same strain marked Ara-. Figure 1 shows the results, where we see strong evidence for marker neutrality. Specifically, the fitness of an Ara+ strain relative to the (otherwise isogenic) Ara- strain is not significantly different than 1 in all cases (one sample t tests, $p > 0.05$).

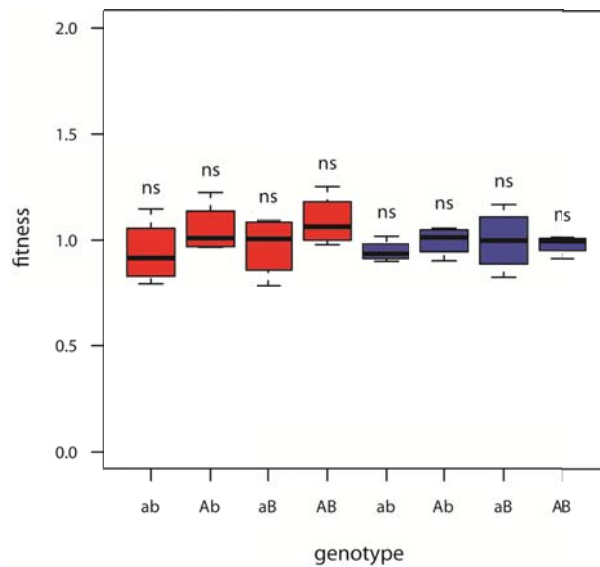


Figure 1: The competitive fitness of an Ara- strain relative to the same strain marked Ara+. The boxes are different engineered genotypes from *Gradual* population 13 (red) and *Gradual* population 18 (blue). Each box is drawn from the first to third quartile with the median as the thick horizontal line. All competitions were done in the absence of rifampicin. The genotype abbreviations are as follows: for *Gradual* population 13, 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, ab (bases a443 and c1527), Ab (bases t443 and c1527), aB (bases a443 and a1527) and AB (bases t443 and a1527). The “ab” genotype from both populations was reconstructed from the “Ab” genotype.