



Figure S2 Example of calibration procedure, showing the evolved *nagC* SNP allele from population Ara-1. We calibrated only those mutations found in the 20,000-generation clone REL8593A. We mixed REL607 cells and evolved REL8593A cells in standardized ratios, and compared measured frequencies to their actual frequencies in the mixture, correcting for differences in average DNA content. From the triplicate assays of REL607/REL8593A mixtures, we fit a calibration curve for each allele to an empirical function that corrected for a symmetric convex bias that captured the deviation from linearity. We used these curves to correct the GenomeStudio estimates of allele frequencies in all of the Ara-1 population samples that we analyzed.