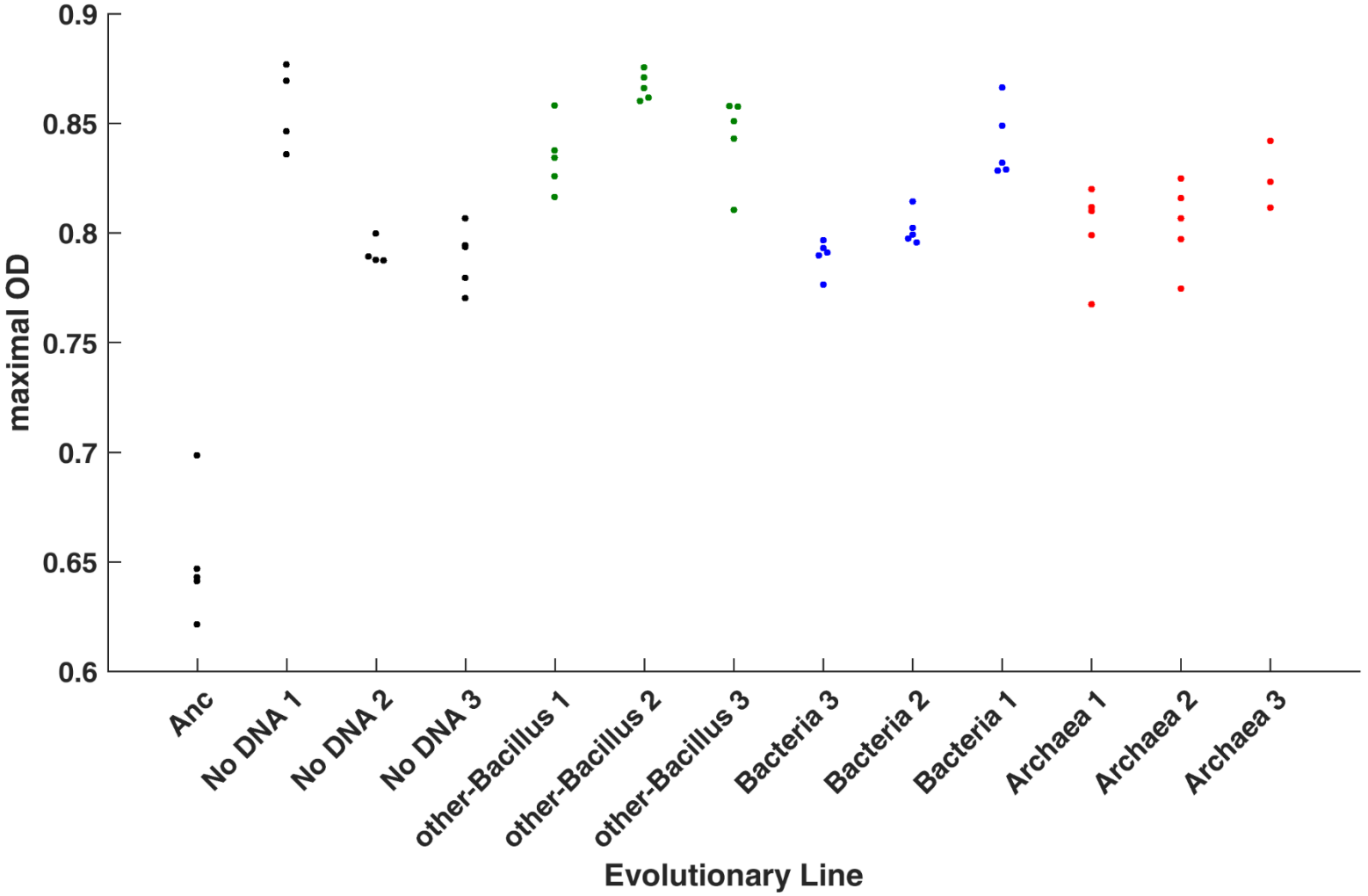
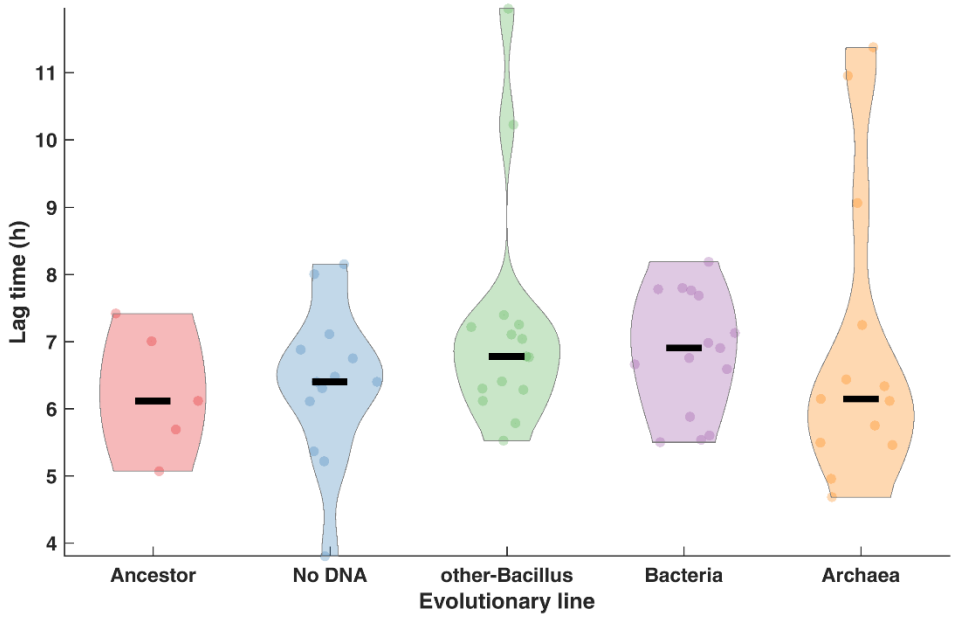


Figure S1: Maximal OD improvement of all 12 evolutionary lines.
Maximal OD values (dots) for each evolutionary line and the ancestor from several growth experiments. Values were obtained using the curveball algorithm (Ram et al. 2019). The number of independent growth experiments performed for each population ranged between three to five. The identity of the population (experiment regime and number of evolutionary line) is depicted on the x-axis; dots are color-coded according to the experiment regime as in Figure 1A.

A


Lag time (h)
11
10
9
8
7
6
5
4
Ancestor
No DNA
other-Bacillus
Bacteria
Archaea
Evolutionary line

B

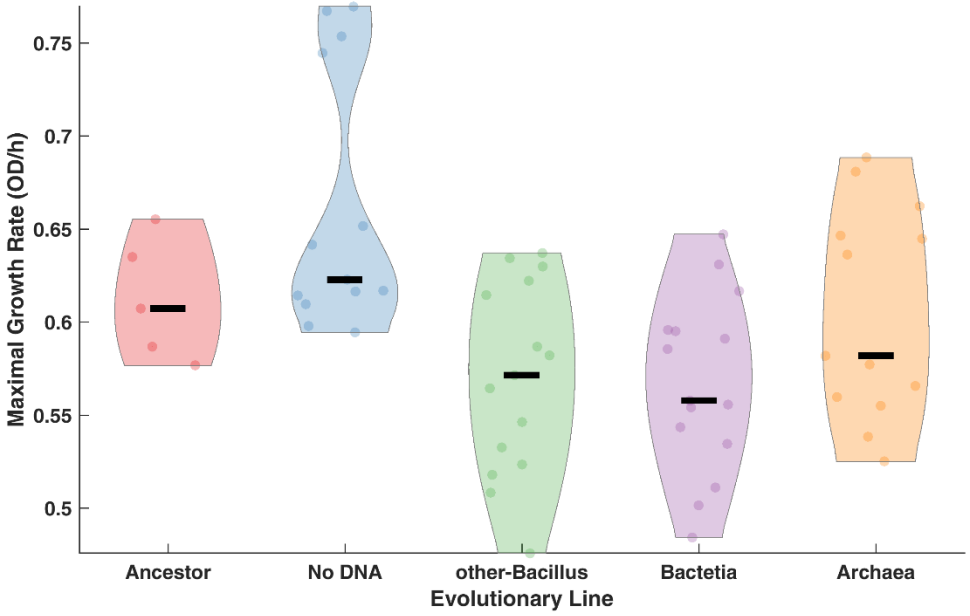

Maximal Growth Rate (OD/h)
0.75
0.7
0.65
0.6
0.55
0.5
Ancestor
No DNA
other-Bacillus
Bactetia
Archaea
Evolutionary Line

Figure S2: Comparison of lag time and maximal growth rate between evolutionary regimes.
Lag time (y axis) (**A**) and maximal growth rate (y axis) (**B**) were extracted from several growth experiments using the curveball algorithm (Ram et al. 2019). The violin plots present the distribution of curveball-derived values for the ancestor as well as each of the four experiment regimes (grouping together all three evolutionary lines, each measured in three to five independent growth experiments). Black lines indicate median values (median, mean and standard deviation values can be found in Table S1). Statistical pairwise comparisons between each evolved group to the ancestor were made (rank sum tests) and no significant results were found.

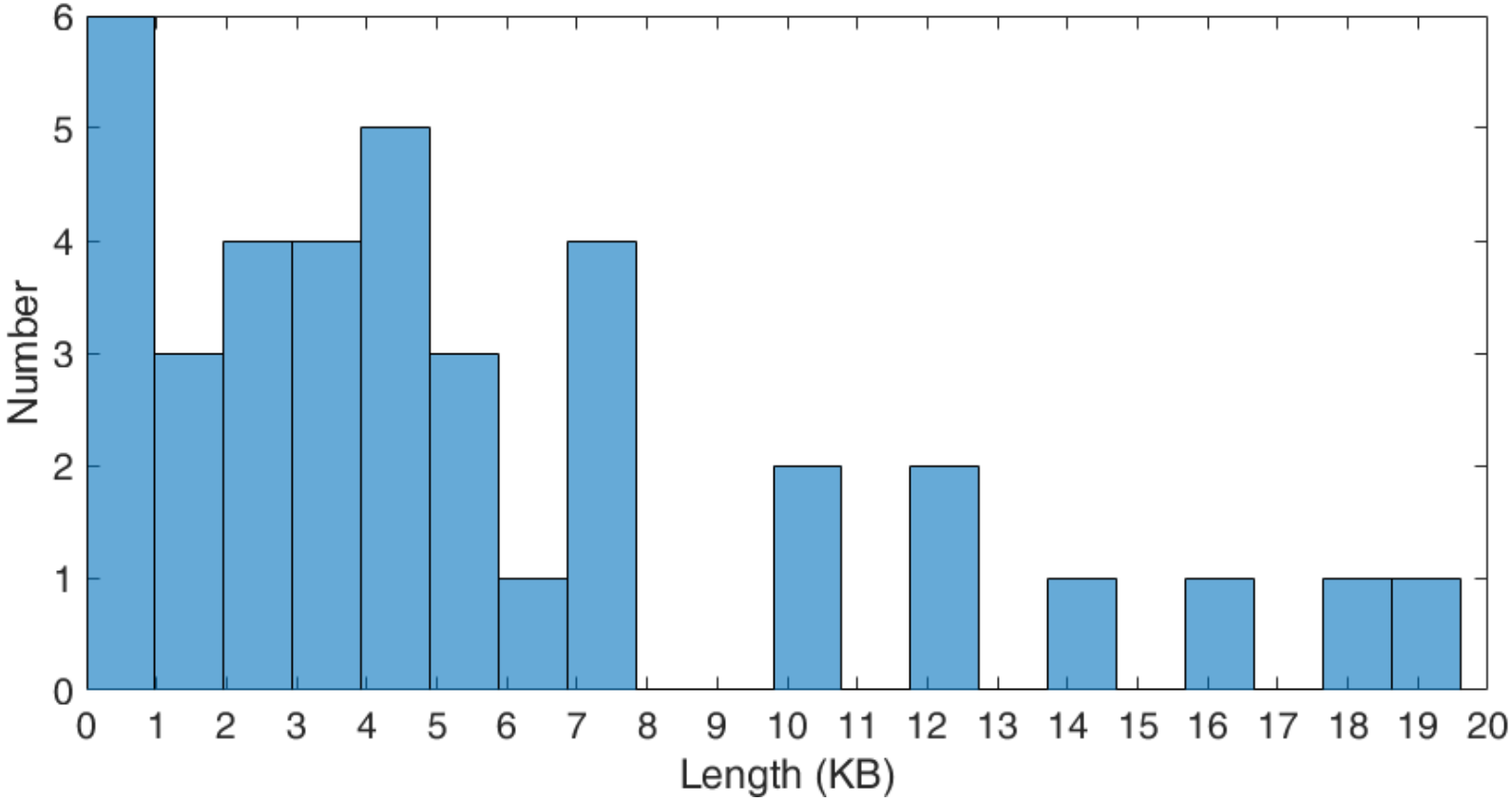


Figure S3: Length distribution of HGT-acquired fragments.
The 38 foreign DNA fragments acquired by all three other-*Bacillus* populations, as identified in generation 504, were binned based on length (bin size 1kb). The number of fragments (y axis) is plotted against the fragment size (x axis).

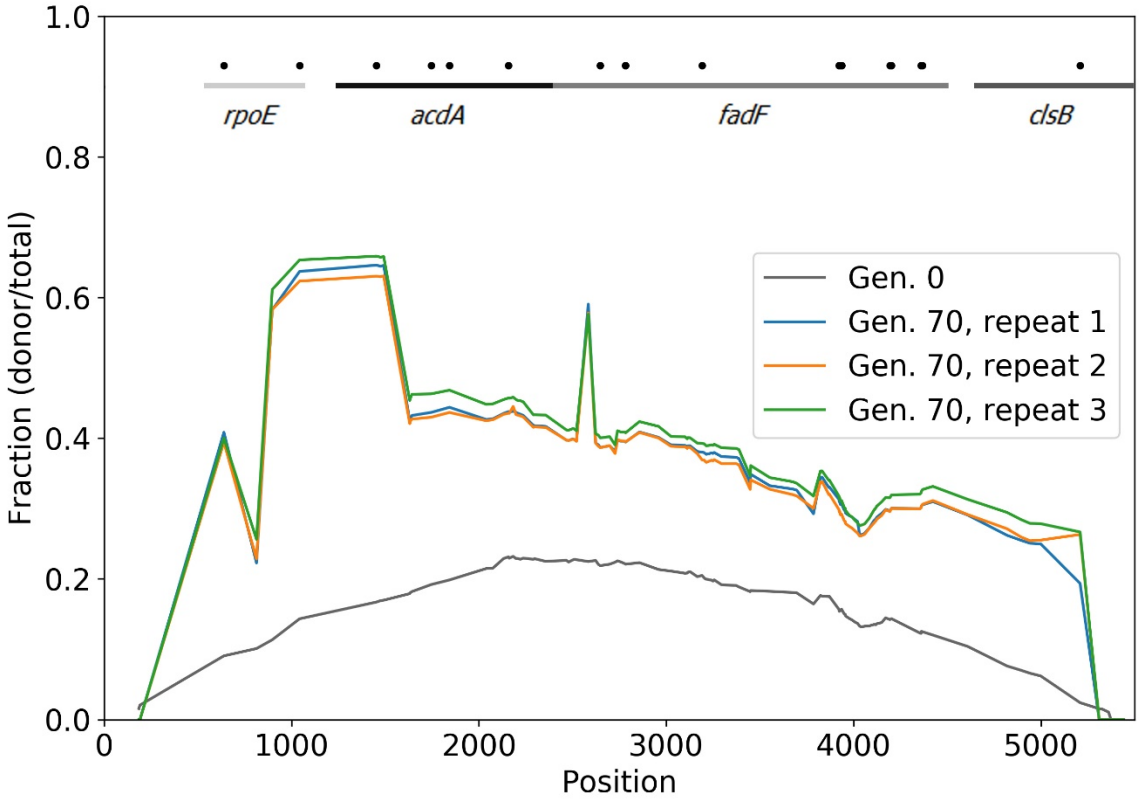


Figure S4: Acquisition of an HGT fragment provides a fitness advantage under LB medium.
Results of three competition assay replicates (70 generations) of a library of transformed *Bacillus subtilis* 168 competent cells, containing different or no regions of a 5.6kb DNA fragment from *Bacillus subtilis* strain RS-D-2, competed under standard salt LB conditions. The graph portrays the frequency of each mismatch along the fragment before and after the competition as calculated based on deep sequencing. The frequency of each mismatch at generation 0 (immediately after transformation) (gray) and in generation 70 (blue, orange and green) is presented on the y-axis. The mismatch location along the fragment is presented on the x-axis. The lines and gene names at the top of the figure denote the genes located in the corresponding regions in the *Bacillus subtilis* 168 genome . Black dots denote mismatches that result in non-synonymous substitutions.

Real Data

Pairwise genomic distances between all fragments

1	2	3	4	…30
	d(2,1)	d(3,1)	d(4,1)	d(30,1)
		d(3,2)	d(4,2)	d(30,2)
			d(4,3)	d(30,3)
				d(30,4)

$$Real\ Proximity\ score = \frac{\#<T}{\#Total\ distances}$$

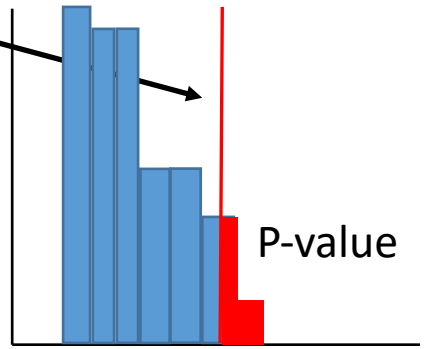
<T
T = distance threshold

Randomized locations data

1	2	3	4	…30
	d(2,1)	d(3,1)	d(4,1)	d(30,1)
		d(3,2)	d(4,2)	d(30,2)
			d(4,3)	d(30,3)
				d(30,4)

X 1000 times

Randomized proximity score distribution



Repeat for different T

Figure S5: Analysis of genomic proximity between integrated HGT fragments.
Presented is a conceptual figure, depicting the process of the genomic proximity analysis performed on the HGT fragments that were detected throughout the evolution in the other-*Bacillus* populations. A distance matrix for the pairwise genomic distances between all 30 fragments was calculated. A distance threshold of T – was set, such that all distances smaller than T were counted and divided by the total number of pairwise distances to serve as a proximity score. Then, the same process was performed, using randomized locations for the HGT fragments, and repeated 1000 time to create a distribution of random proximity scores. The p value of genomic proximity of the HGT fragments at a given value of T, was set as the proportion of randomized scores that are equal or larger than the real score (in red). This procedure was repeated for different threshold values of T (1%, 0.5% and 0.25% of half of the length of the genome of *B. subtilis* 168, corresponding to 21078bp, 10539bp and 5269bp, respectively).

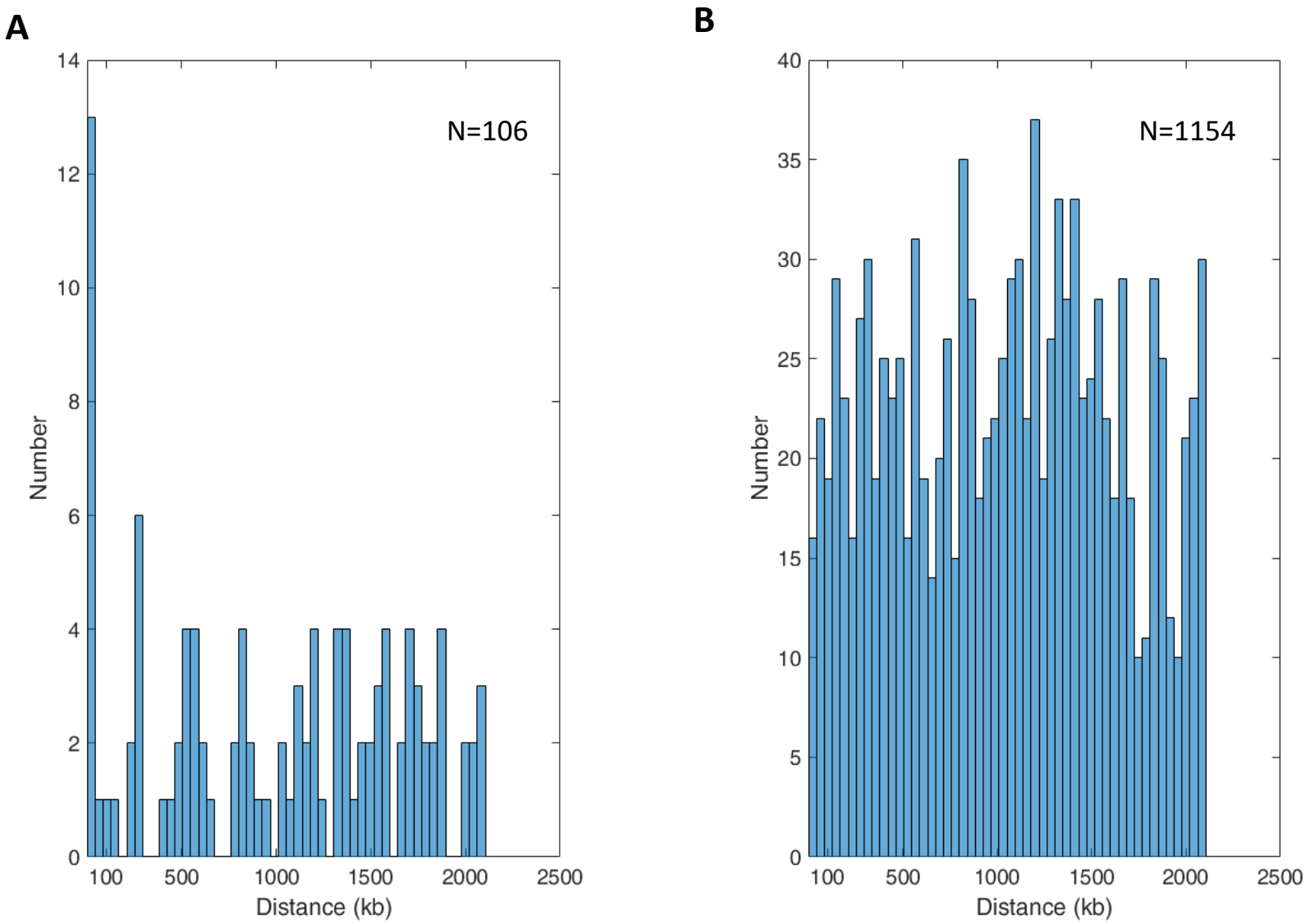


Figure S6: pairwise genomic distance distribution between mutations and HGT fragments observed in the other-*Bacillus* populations. Distribution of distances between mutations and HGT fragments residing in the same clone (**A**) and in different clones (**B**), based on calculations of the pairwise distance between mutations and HGT fragments. N indicates the total number of pairwise distances in each distribution.