

Supplemental information for the paper:

**Changes in Cell Size and Shape During 50,000 Generations  
of Experimental Evolution with *Escherichia coli***

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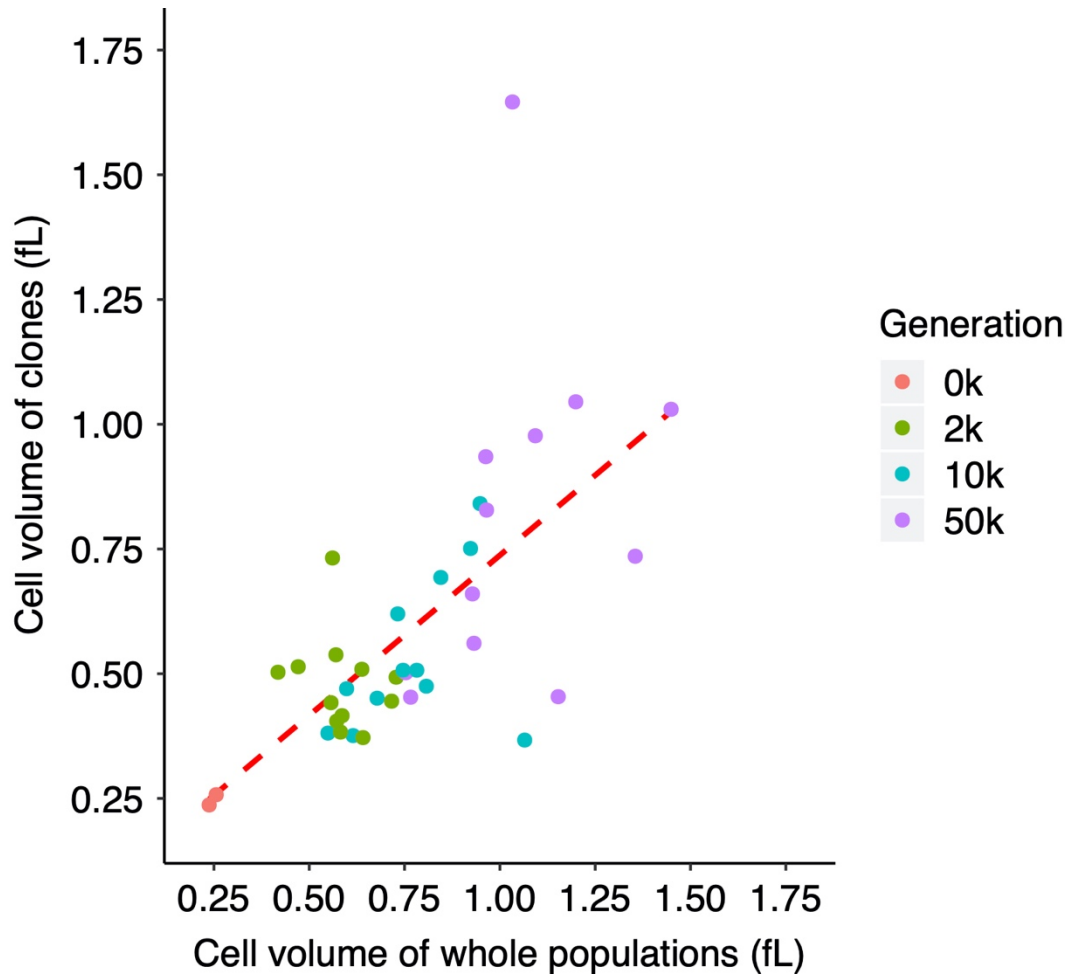
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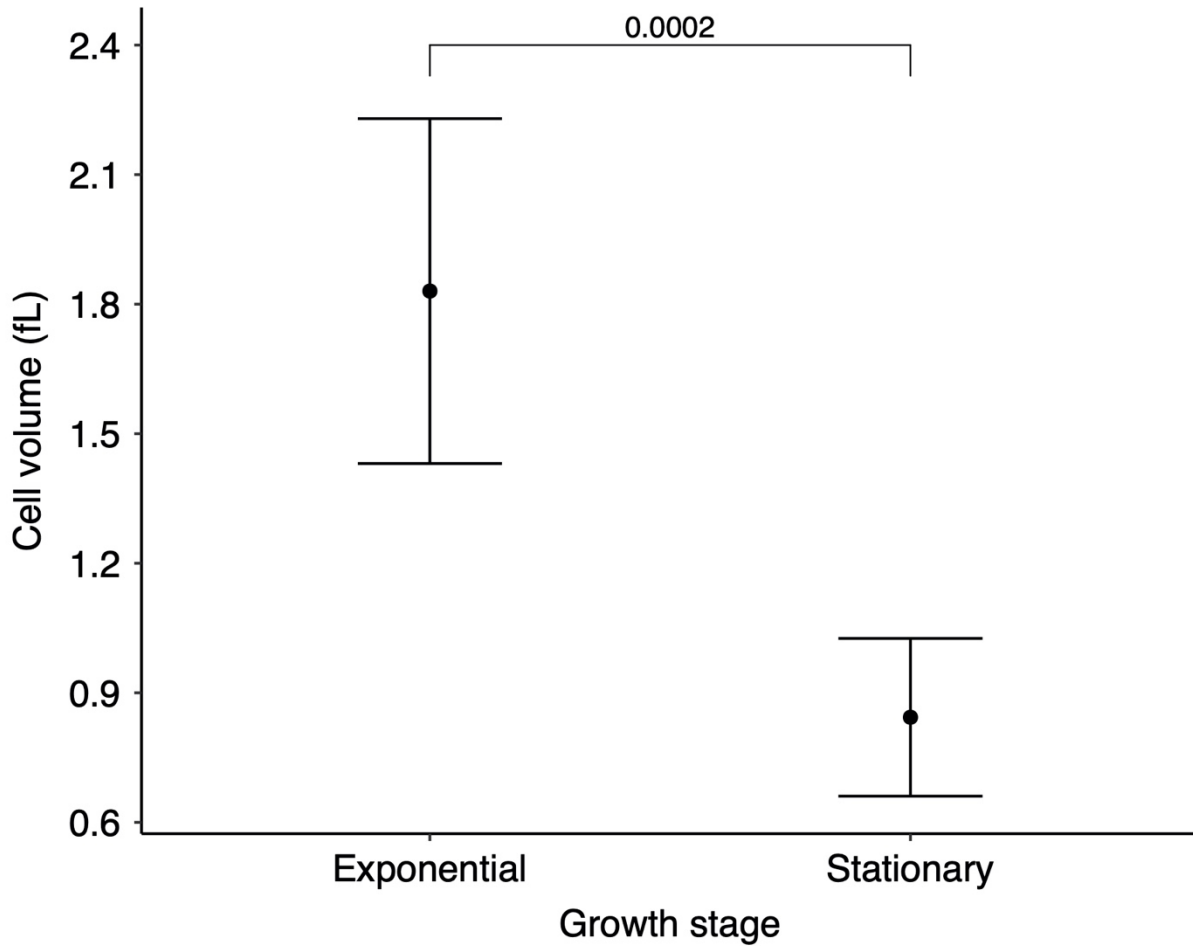
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4 **FIG S1.** Correlation between cell volumes of clones and whole-population samples. The

5 clone and population values are the medians from FIG 2A and FIG 2B, respectively.

6 Kendall's coefficient  $\tau = 0.4900$ ,  $N = 38$ ,  $p < 0.0001$ .

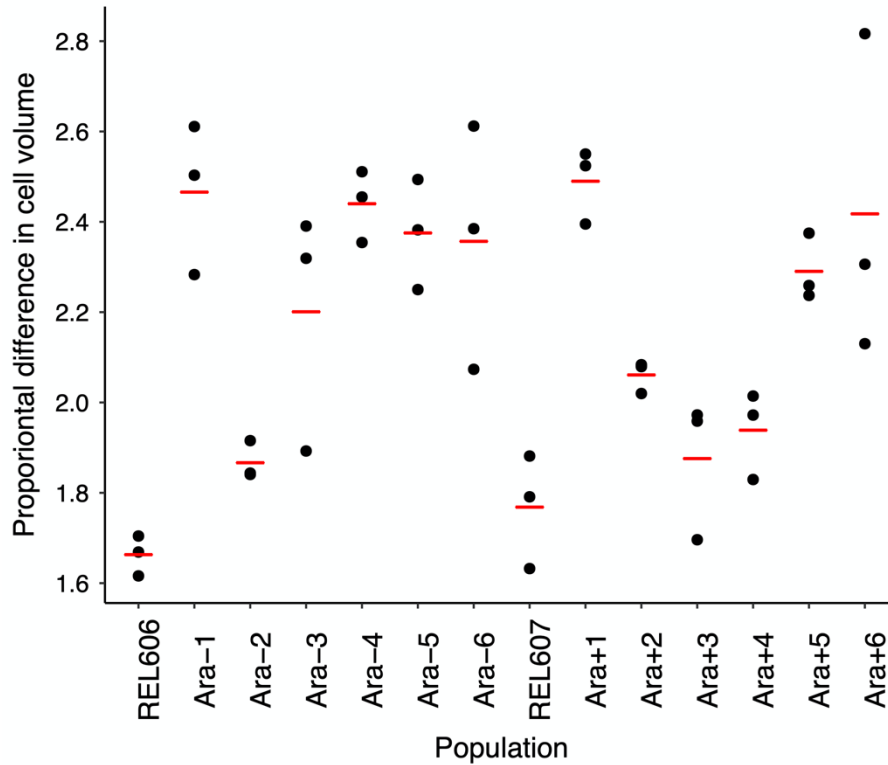
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10 **FIG S2.** Difference in cell size between exponential and stationary phases. We calculated  
11 the grand median cell volume of each 50,000-generation clone grown for 2 h (exponential)  
12 or 24 h (stationary), and then computed the grand mean of the 12 populations. Error bars  
13 are 95% confidence intervals, and the bracket shows the statistical significance ( $p$  value)  
14 based on a one-tailed Wilcoxon test, which accounts for the paired nature of the samples.



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16 **FIG S3.** Proportional difference in volume between exponentially growing and stationary-

17 phase cells. The y-axis shows ratio of the volume of exponentially growing and stationary-

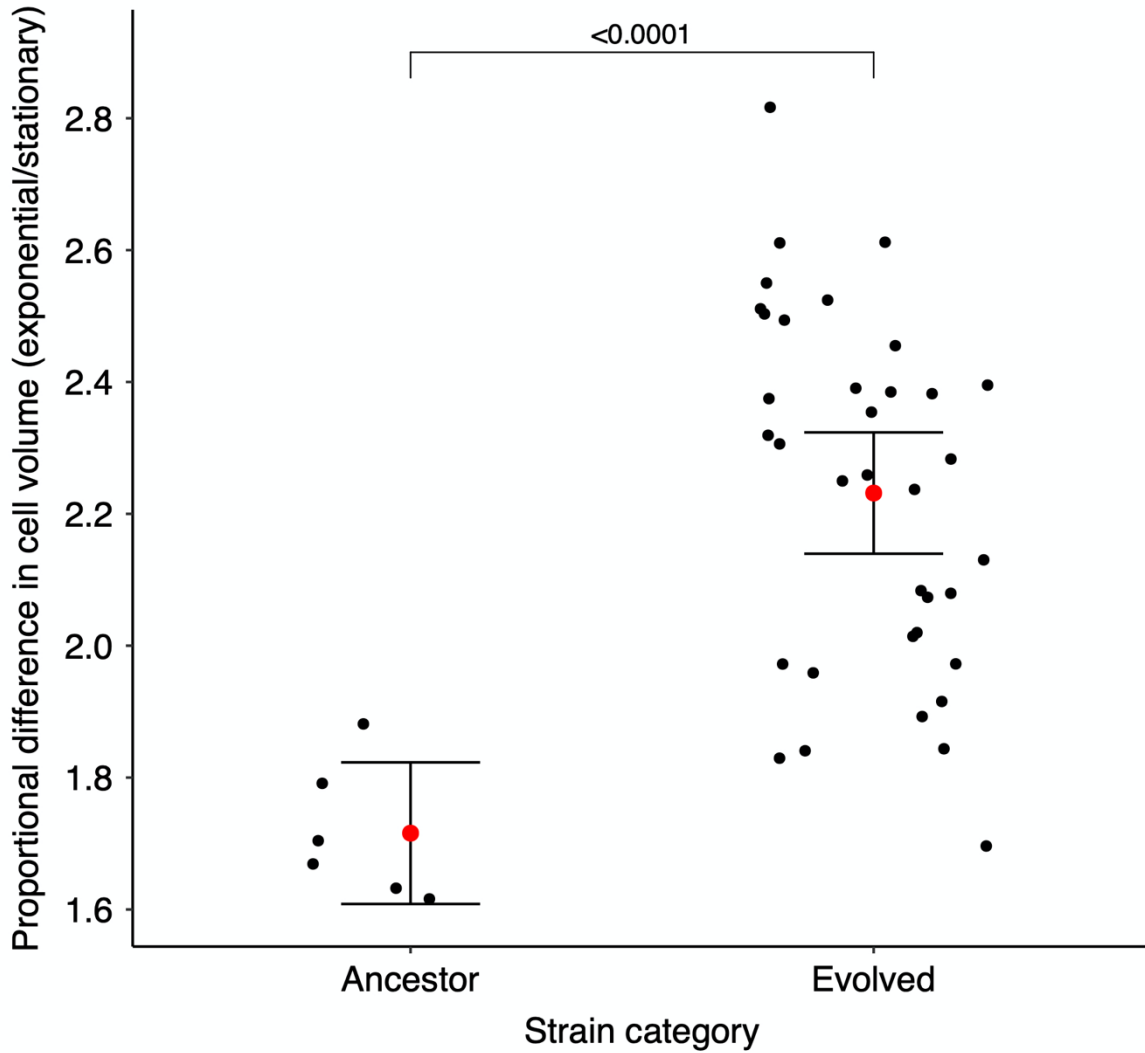
18 phase cells for the ancestors (REL606 and REL607) and clones from generation 50,000.

19 Data were collected using a Coulter counter. Each black circle shows the ratio of median

20 cell volumes for a pair of assays in the two growth states. Red bars show the means of

21 the 3 paired assays for each strain.

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24 **FIG S4.** Proportional difference in cell volumes of ancestors and evolved clones during

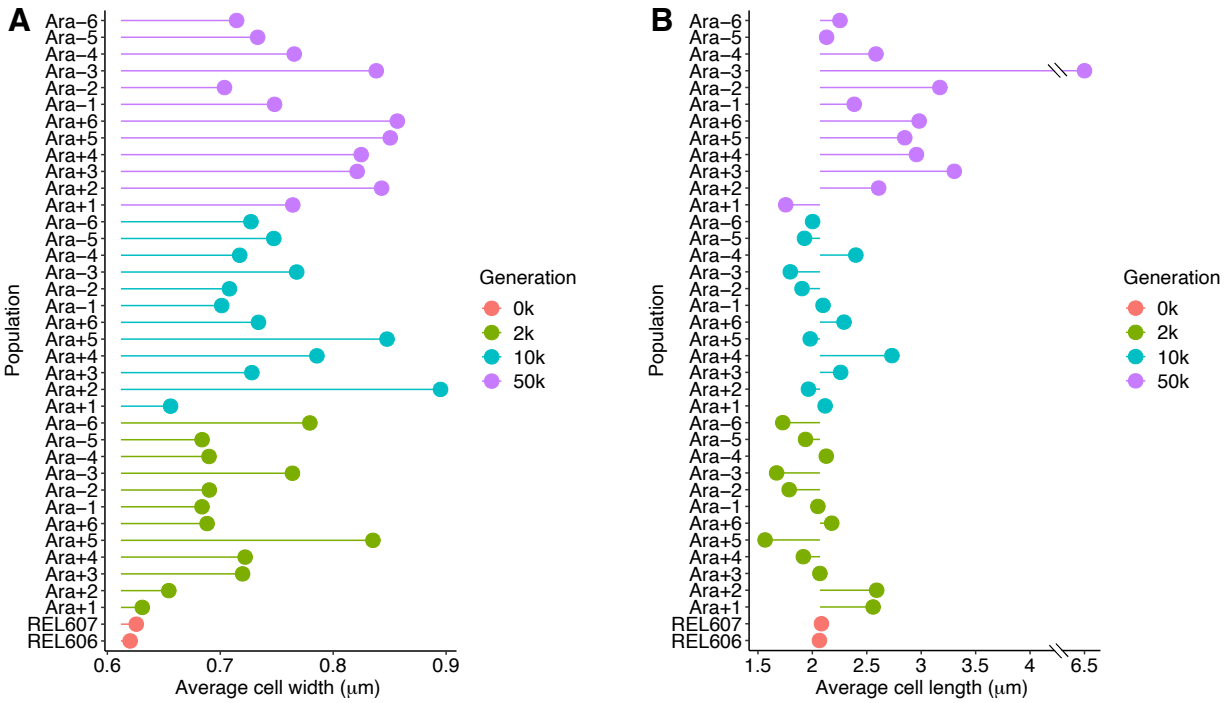
25 exponential and stationary phases. Data and methods from FIG S3. Black points show

26 estimates for each replicate and strain, with scatter along the x-axis for visual clarity; red

27 points are the grand means. Error bars are 95% confidence intervals, and the bracket

28 above shows the statistical significance ( $p$  value) based on a one-tailed unpaired  $t$ -test.

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32 **FIG S5.** Average cell widths (A) and lengths (B) for the ancestor and evolved samples.

33 Cell widths and lengths were calculated by multiplying the ShortAxis and LongAxis

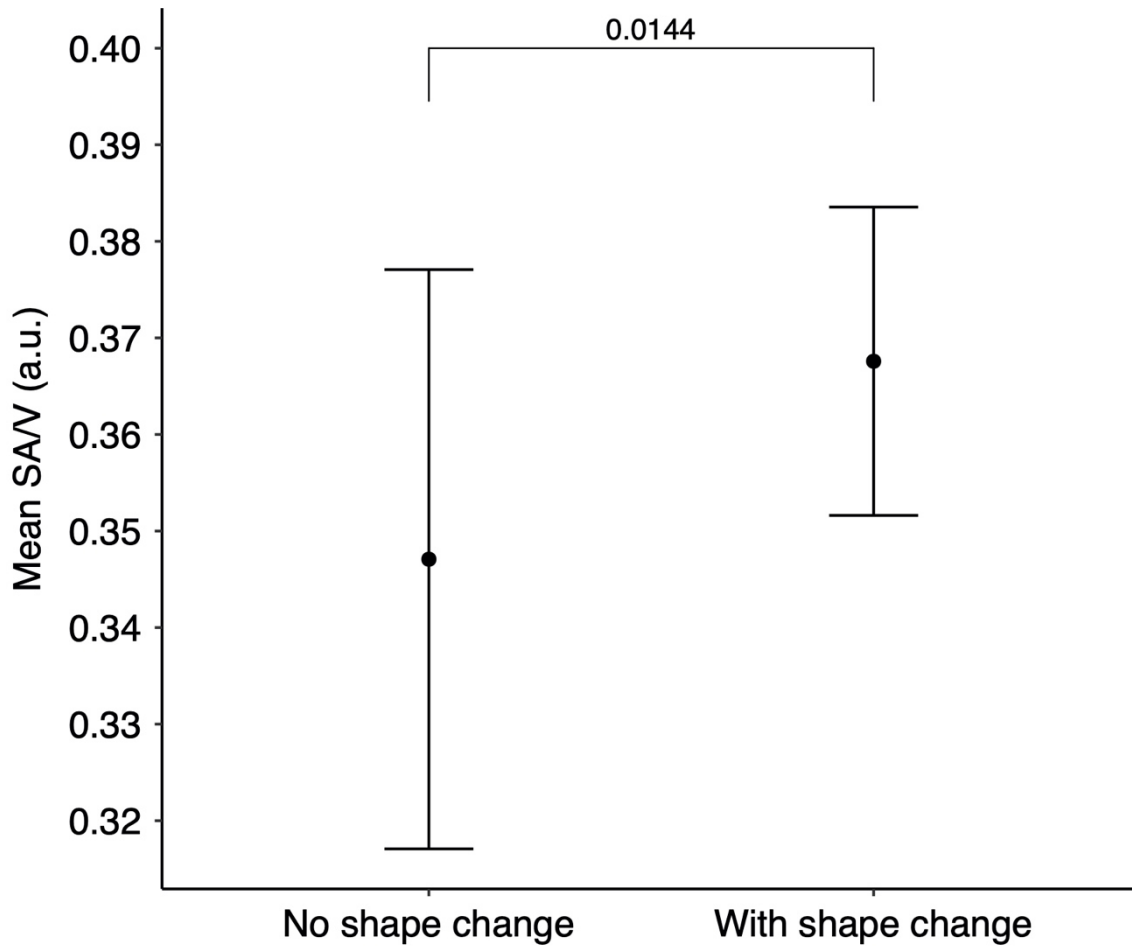
34 measurements (both in unit pixel) from the *SuperSegger* output by the conversion factor

35 of  $0.0664 \mu\text{m}/\text{pixel}$ . The means were calculated from three replicate assays in all but 4

36 cases (Ara-4 at 10,000 generations; Ara-2, Ara-4, and Ara-5 at 50,000 generations),

37 which had two replicates each.

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42 **FIG S6.** Isometric analysis of the surface area-to-volume ratio (SA/V) to assess the effect  
43 of the change in aspect ratio (length/width) from 10,000 to 50,000 generations. For each  
44 population, we calculated a hypothetical mean SA/V using its average aspect ratio at  
45 10,000 generations (no shape change), and we compared it to a hypothetical mean SA/V  
46 calculated using its average aspect ratio at 50,000 generations (with shape change). This

47 latter value differs from that shown in FIG 10, because that figure is based on direct  
48 measurements of individual cells, followed by averaging the cells in a single assay,  
49 averaging the replicate assays for each population, and calculating the grand mean of the  
50 12 populations. By contrast, calculating a hypothetical mean SA/V for a population using  
51 its aspect ratio from a different generation can only use average values of the relevant  
52 parameters. Therefore, we applied the same population-level averages to compare ratios  
53 with and without shape changes here. Error bars are 95% confidence intervals, and the  
54 bracket shows the statistical significance ( $p$  value) based on a one-tailed paired  $t$ -test.  
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**Table S1:** List of *E. coli* clones used in study

<b>Clone ID</b>	<b>Population</b>	<b>Generation</b>
REL606	Ara <sup>-</sup> ancestor	0
REL607	Ara <sup>+</sup> ancestor	0
REL1158A	Ara+1	2,000
REL1159A	Ara+2	2,000
REL1160A	Ara+3	2,000
REL1161A	Ara+4	2,000
REL1162A	Ara+5	2,000
REL1163A	Ara+6	2,000
REL1164A	Ara-1	2,000
REL1165A	Ara-2	2,000
REL1166A	Ara-3	2,000
REL1167A	Ara-4	2,000
REL1168A	Ara-5	2,000
REL1169A	Ara-6	2,000
REL4530A	Ara+1	10,000
REL4531A	Ara+2	10,000
REL4532A	Ara+3	10,000
REL4533A	Ara+4	10,000
REL4534A	Ara+5	10,000
REL4535A	Ara+6	10,000

REL4536A	Ara-1	10,000
REL4537A	Ara-2	10,000
REL4538A	Ara-3	10,000
REL4539A	Ara-4	10,000
REL4540A	Ara-5	10,000
REL4541A	Ara-6	10,000
REL11392	Ara+1	50,000
REL11342	Ara+2	50,000
REL11345	Ara+3	50,000
REL11348	Ara+4	50,000
REL11367	Ara+5	50,000
REL11370	Ara+6	50,000
REL11330	Ara-1	50,000
REL11333	Ara-2	50,000
REL11364	Ara-3	50,000
REL11336	Ara-4	50,000
REL11339	Ara-5	50,000
REL11389	Ara-6	50,000

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**Table S2:** List of *E. coli* whole-population samples used in study

<b>Sample ID</b>	<b>Population</b>	<b>Generation</b>
REL1158	Ara+1	2,000
REL1159	Ara+2	2,000
REL1160	Ara+3	2,000
REL1161	Ara+4	2,000
REL1162	Ara+5	2,000
REL1163	Ara+6	2,000
REL1164	Ara-1	2,000
REL1165	Ara-2	2,000
REL1166	Ara-3	2,000
REL1167	Ara-4	2,000
REL1168	Ara-5	2,000
REL1169	Ara-6	2,000
REL4530	Ara+1	10,000
REL4531	Ara+2	10,000
REL4532	Ara+3	10,000
REL4533	Ara+4	10,000
REL4534	Ara+5	10,000
REL4535	Ara+6	10,000
REL4536	Ara-1	10,000
REL4537	Ara-2	10,000

REL4538	Ara-3	10,000
REL4539	Ara-4	10,000
REL4540	Ara-5	10,000
REL4541	Ara-6	10,000
REL11383	Ara+1	50,000
REL11325	Ara+2	50,000
REL11326	Ara+3	50,000
REL11327	Ara+4	50,000
REL11362	Ara+5	50,000
REL11363	Ara+6	50,000
REL11318	Ara-1	50,000
REL11319	Ara-2	50,000
REL11354	Ara-3	50,000
REL11321	Ara-4	50,000
REL11322	Ara-5	50,000
REL11382	Ara-6	50,000

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