# Supporting Information

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## SI Materials and Methods

**Estimation of Relative Fitness.** Briefly, both competitors (the Ara- evolved line and the ancestral Ara+ REL1207 strain) were revived in Luria–Bertani (LB) broth and grown separately for 1 d at 37 °C and a second day at the temperature of interest (20 °C or 37 °C) in 10 mL DM25. Then the two competitors were mixed at a 1:1 volumetric ratio and diluted 100-fold into 10 mL fresh DM25 and incubated 1 d at the assay temperature. The initial and final densities were estimated by plating the culture onto tetrazolium-arabinose (TA) agar plates.

**Estimation of Absolute Fitness.** Briefly, the assays began by reviving the clones in 1 mL LB and incubating overnight at 37 °C with constant shaking (100 rpm). The following day, cultures were diluted 100-fold into $MgSO_4$ (10 mM solution) and 10 μl of this dilution was transferred into 990 μl DM25. The 96-well plates (Megatiter plates, Neptune) were covered with gas permeable seals (Thermo Scientific) and incubated 1 d at 37 °C (day 0) to allow the clones to acclimate to culture conditions. The following days (days 1–3), we transferred 10 μl of the overnight culture in 990 μl of fresh DM25 (100-fold dilution) and we incubated the plates at the assay temperature. The plates were incubated in a shaking incubator (Innova 4300) with an accuracy of ±0.25 °C. At the end of each day we measured population densities using an electronic particle counter (Coulter Counter model Multisizer 3 equipped with a 30-μm-diameter aperture tube). To measure density, 50 μl of culture was diluted in 9.9 mL IsotonII diluent (Beckman Coulter), and 50 μl of the resulting dilution was counted electronically. We subtracted the background noise to the counts by measuring the number of particle in 50 μl sterile DM25. Our pilot study showed a high correlation between the densities estimated with viable cell counts (colony-forming units) and estimated with electronic counts ($R^2 = 0.867$, $P = 7.55^{-15***}$).

To estimate the absolute fitness we used a fixed effects linear model, in which we defined the natural logarithm of the cell density as the dependent variable, and the time (daily transfers) as the independent variable. The cell density from the first day of acclimation (day 0) was excluded from the regression.

Let $y^l_{r,d}$ be the natural logarithm of the bacterial density, in which $d$ is the day of transfer, $l$ is the line analyzed (high-temperature adapted clones or the ancestor), and $r$ is the replicate. We used the following model:

$$y^l_{r,d} = \left(\beta_{o,1} * 1_{r=1} + \beta_{o,2} * 1_{r=2} + \beta_{o,3} * 1_{r=3}\right) + \beta_1 * d + \varepsilon^l_{r,d},$$

where $\beta_0$ is the intercept for each replicate, $\beta_1$ is the regression coefficient, and $\varepsilon^l_{r,d}$ is the error term. The slope of the fitted linear regression $\beta_1$ is equivalent to the absolute fitness of a given genotype at a given temperature.

**Strain Construction and Confirmation of Recombinants.** We used the pJk611 recombineering plasmid, kindly provided by M. Raffatellu, Department of Microbiology and Molecular Genetics, University of California, Irvine, CA. The pJk611 plasmid is identical to the pkD46 plasmid, with the addition of a *sacB* gene used to eliminate the plasmid when counterselected on LB with sucrose.

Briefly, we first introduced the pJk611 plasmid into the ancestral strain, electroporating 2 μl plasmid (containing between 0.5 and 1 μg plasmid) into 50 μl of competent cells using an Eppendorf Electroporator 2510 set at 1.8 kV. Following electroporation, we added 1 mL LB and incubated the cells at 30 °C for 2 h with shaking. We then plated 100 μl of cells on LB agar plates containing 100 μg/mL ampicillin to select ampicillin-resistant ($amp^R$) transformants. The ancestral strain carrying the pJk611 plasmid was then grown overnight at room temperature (~20 °C) in 25 mL LB with 100 μg/mL ampicillin and 1 mM L-arabinose (Sigma) until it reached an $OD_{600}$ of 0.6. We made electrocompetent cells by washing the cultures five times with ice-cold water. We simultaneously introduce two oligos of 70 bp with the desired nucleotide change in the center of the oligo (Table S3). The first oligo was used to introduce single mutation in *rpoB* or *rho*; the second oligo was to introduce the mutation that produces the Ara+ phenotype. Two μl of each oligo (10 μM) was electroporated into 50 μl of cells. After electroporation we added 1 mL LB and incubated cells at 37 °C for 3 h with shaking and spread 500 μl in minimal medium agar (MA) plates supplemented with L-arabinose. The remainder was grown overnight at room temperature and 100 μl was spread in MA plates. The plates were incubated 48 h at 42 °C. We selected 94 single colonies and streaked them onto TA agar plates, incubated overnight at 37 °C. We screened for the mutations by doing PCR on single colonies to amplify the region of the *rpoB* or *rho* gene with the mutation and Sanger sequencing the fragments of ~370 bp (Table S3). The PCR thermal cycling conditions were 94 °C for 10 min followed by 30 cycles of 94 °C 20 s, 60 °C 30 s, and 68 °C 1 min; finally 68 °C for 5 min.

**Genetic Associations.** We first created a list of unique mutations (these mutations can be any kind of molecular change, from point mutations to large deletions) occurring in 114 high-temperature adapted clones. We then selected five of the most commonly shared mutations (mutations shared by 14 or more clones), which represent a disjoint set at the right tail of the frequency distribution (Fig. S3), to explore the effect of each mutation on the absolute fitness (Table 1).

We selected $L_m$, which is the list of the high-temperature adapted clones in which the mutation $m$ occurred (see Fig. S7 for a simplified example). We then found all of the mutations occurring in $L_m$, denoted by $M_m$ (including $m$), and included them in the analysis.

Except for the mutations that are shared in more than one clone, most of the mutations are cooccurring in the same clone, which prevents us from drawing conclusions regarding the effect of single mutations on the phenotype. To overcome this problem, we partitioned the mutations into clusters $C$. A cluster is a subset of mutations that always occur together, and it contains a mutation $m$ if and only if $m \in C$ for all lines in $L_m$. If a mutation has no cooccurring mutation, then it forms its own cluster. We created a cluster incidence matrix showing the presence or absence of a cluster in a clone (Fig. S7). We then combined the cluster incidence matrix with the continuous data of absolute fitness at 18 °C to fit the following fixed effects linear model:

$$y^l_{r,d} = \left(\beta_0^{l=1} * 1_{l=1} + \ldots + \beta_0^{l=n} * 1_{l=n}\right) + \beta_1 * d + \left(\beta_{1,m_1} * d * 1_{m1} + \ldots + \beta_{1,m_M} * d * 1_{m_M}\right) + \varepsilon^l_{r,d},$$

where $m_1,\ldots,m_M$ are the groups of mutations that are being analyzed. In our model, $\beta_0^{l=1} * 1_{l=1} + \ldots + \beta_0^{l=n} * 1_{l=n}$ are the interceptions coefficients associate with each clone, $\beta_1$ is the absolute fitness of the overall population (all clones in $L_m$) without the effect of the mutations $m_1$ to $m_M$. In our model, the effect of the presence of the mutation $m_i$ on the absolute fitness is given by the coefficient $\beta_{1,m_i}$.

In short, the model fitted a regression line for each one of the clones, where the slope of the regression for a given clone is given by the sum of the individual slope coefficient of the occurring mutations in that clone.

Finally, for each one of the most commonly shared mutations we selected a final model based on the lowest Akaike Information Criterion and goodness of fit to obtain a parsimonious model that quantifies the contribution of each mutation group to the overall population fitness.

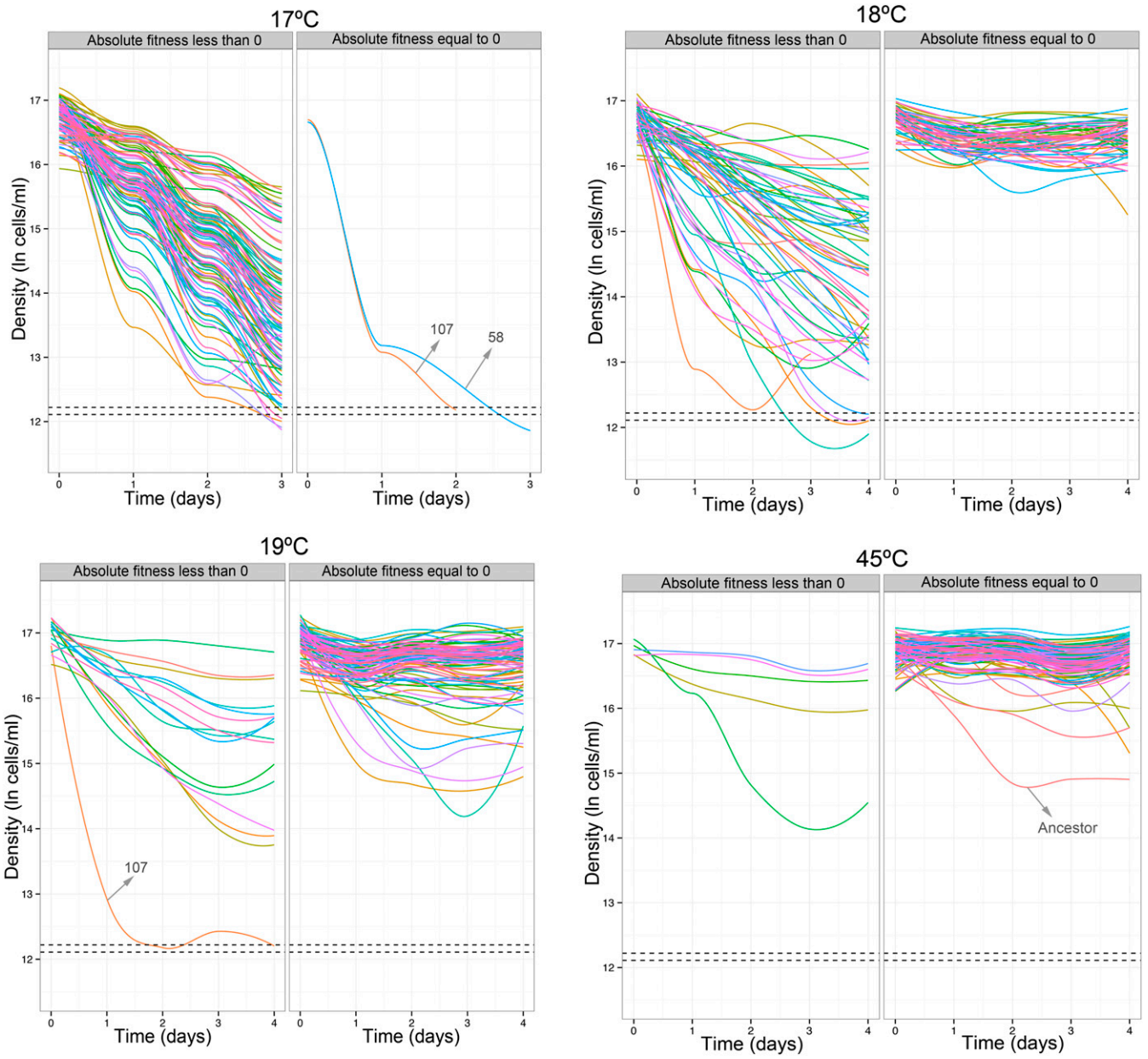



**Fig. S1.** Population density trajectories of the 114 high-temperature adapted clones and the ancestor at 17 °C, 18 °C, 19 °C, and 45 °C. For each temperature, the clones are divided in two groups: (*Left*), which contains the clones in which the slope of the regression is significantly less than zero; and (*Right*), which contains the clones in which the slope of the regression is not significantly different from zero (Table S2). Each line corresponds to a local polynomial regression fitting of three replicates. The two horizontal dotted lines correspond to background noise (SE limits estimated from the mean number of particles present in 71 samples of sterile DM25). Populations crossing the dotted lines are considered extinct. When the background noise was higher than the population density (negative value), the point was excluded from the analysis.

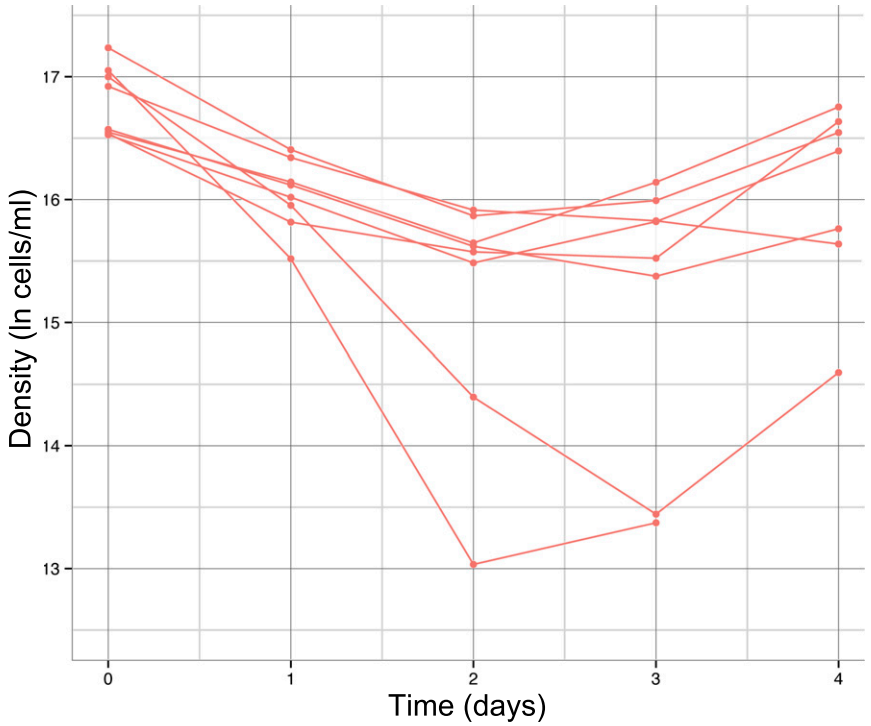



**Fig. S2.** Lazarus effect observed in the ancestor at 45 °C. Each point corresponds to the bacterial density measured at the end of a growth cycle (24 h). The eight solid lines connecting the circles correspond to eight replicate measurements of the ancestor at 45 °C.

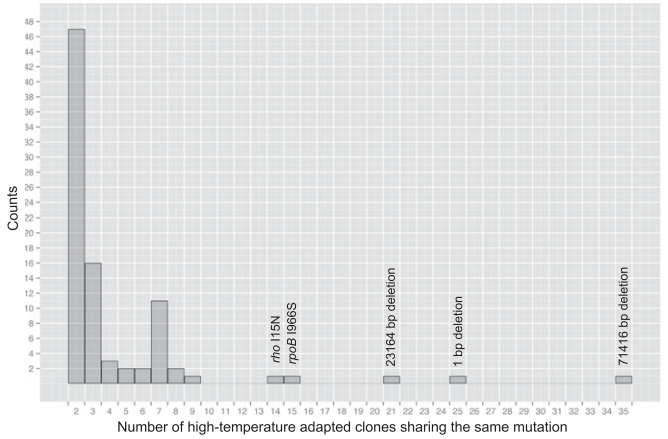



**Fig. S3.** Distribution of the number of high-temperature adapted clones sharing the same identical mutation.

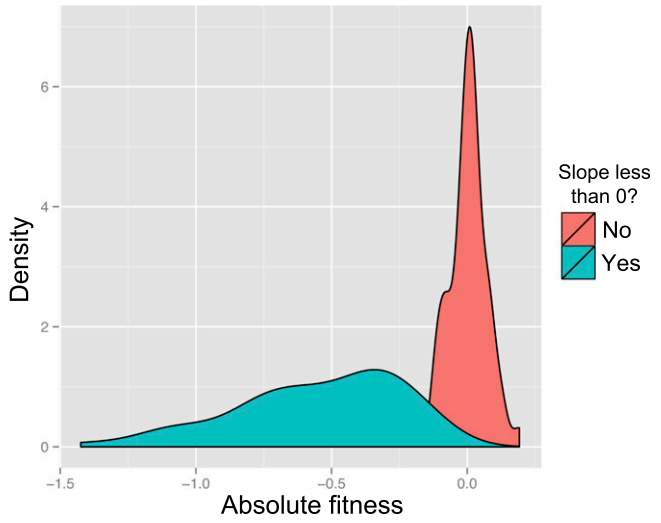



**Fig. S4.** Kernel density plot from the absolute fitness data at 18 °C. The area under the curve represents the empirical probability of occurrence of an absolute fitness value at 18 °C, for the population of 114 high-temperature adapted clones.

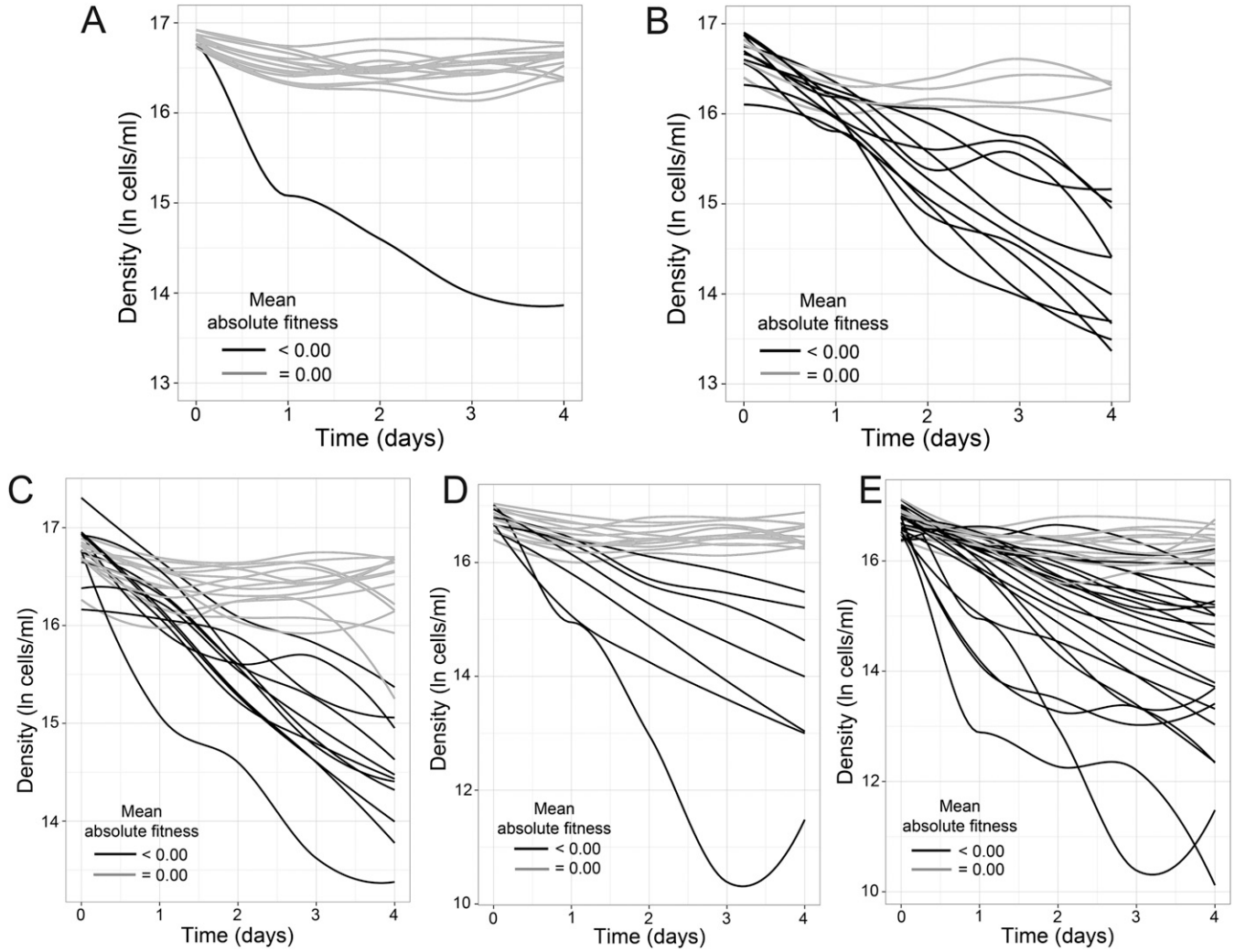



**Fig. S5.** Population density trajectories of the high-temperature evolved clones with the (*A*) *rho* I15N mutation, (*B*) *rpoB* I966S mutation, (*C*) 1-bp deletion, (*D*) 23164 bp large deletion, and (*E*) 71416 bp large deletion.

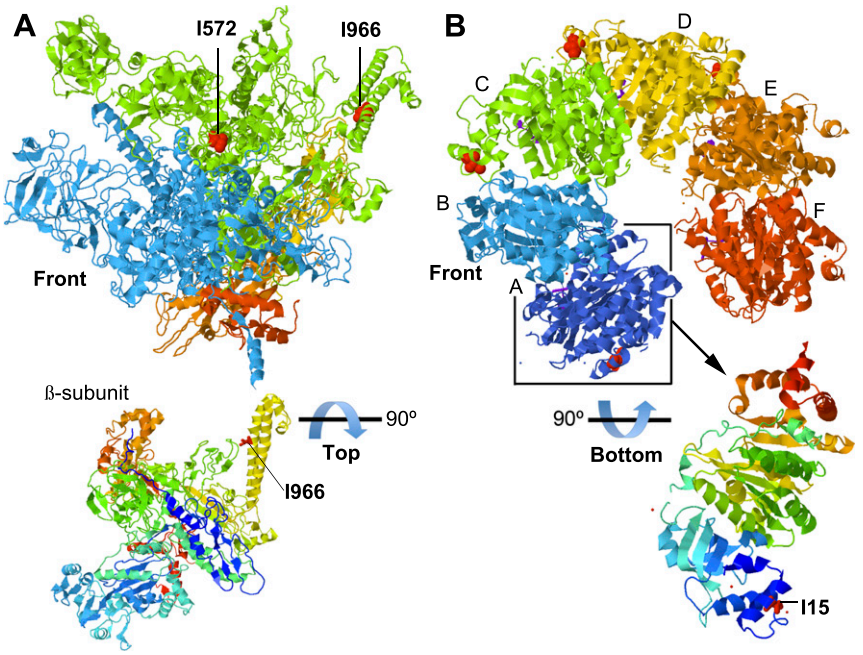
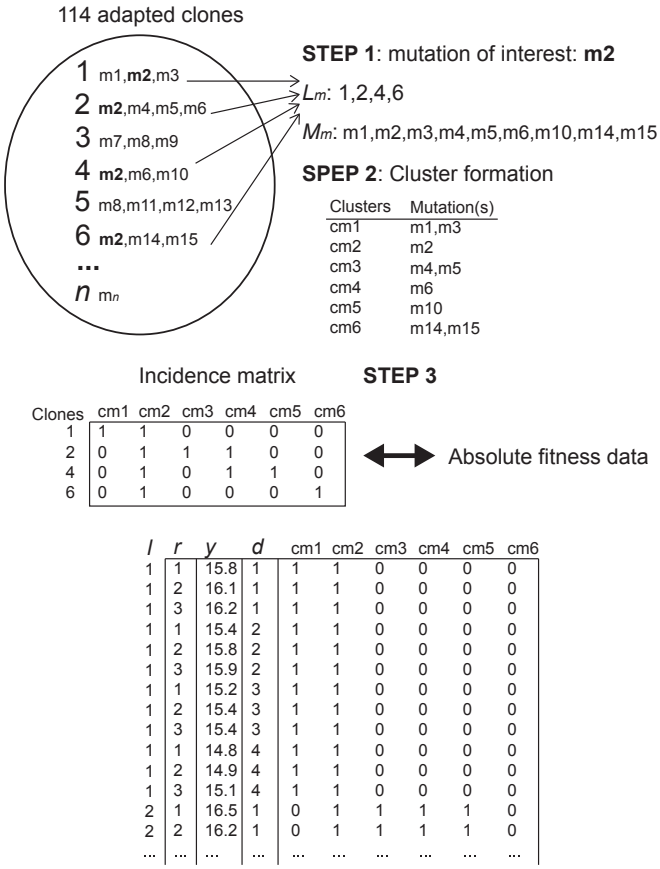



**Fig. S6.** Structural mapping of the codons analyzed. (*A*) Front view of the RNAP from *Escherichia coli* [Protein Data Bank (PDB) ID code 3LUO], color-coded as follows: αI and αII, yellow; β, green; β′, blue; ω, orange. Residues I572 (in the active site of the RNAP) and I966 (at the base of the flap domain) are shown as red spheres. Below is the top view of a ribbon diagram of the β-subunit colored from the N terminus (blue) to the C terminus (red) using a rainbow color gradient. Solvent-exposed residues in the back face of the ladder, including residue I966, are believed to interact with an unidentified regulatory factor. (*B*) Front view of the Rho hexamer from *E. coli* (PDB ID code 1PV4). Residue I15 is shown as red sphere. Below is the bottom view of a Rho protomer colored from the N terminus (blue) to the C terminus (red) using a rainbow color gradient.




| *l* | *r* | *y* | *d* | cm1 | cm2 | cm3 | cm4 | cm5 | cm6 |
|---|---|---|---|---|---|---|---|---|---|
| 1 | 1 | 15.8 | 1 | 1 | 1 | 0 | 0 | 0 | 0 |
| 1 | 2 | 16.1 | 1 | 1 | 1 | 0 | 0 | 0 | 0 |
| 1 | 3 | 16.2 | 1 | 1 | 1 | 0 | 0 | 0 | 0 |
| 1 | 1 | 15.4 | 2 | 1 | 1 | 0 | 0 | 0 | 0 |
| 1 | 2 | 15.8 | 2 | 1 | 1 | 0 | 0 | 0 | 0 |
| 1 | 3 | 15.9 | 2 | 1 | 1 | 0 | 0 | 0 | 0 |
| 1 | 1 | 15.2 | 3 | 1 | 1 | 0 | 0 | 0 | 0 |
| 1 | 2 | 15.4 | 3 | 1 | 1 | 0 | 0 | 0 | 0 |
| 1 | 3 | 15.4 | 3 | 1 | 1 | 0 | 0 | 0 | 0 |
| 1 | 1 | 14.8 | 4 | 1 | 1 | 0 | 0 | 0 | 0 |
| 1 | 2 | 14.9 | 4 | 1 | 1 | 0 | 0 | 0 | 0 |
| 1 | 3 | 15.1 | 4 | 1 | 1 | 0 | 0 | 0 | 0 |
| 2 | 1 | 16.5 | 1 | 0 | 1 | 1 | 1 | 1 | 0 |
| 2 | 2 | 16.2 | 1 | 0 | 1 | 1 | 1 | 1 | 0 |
| ... | ... | ... | ... | ... | ... | ... | ... | ... | ... |

**Fig. S7.** Schematic representation of the procedure followed to prepare the data that were analyzed in the genetic association study. In this simplified example, only six high-temperature adapted clones with few mutations are considered. The first step consists of the selection of the mutations and the high-temperature clones to be analyzed. The second step consists of forming clusters of mutations cooccurring in the same clone. In the last step, the mutation clusters incidence matrix is combined with the absolute fitness data, containing the values of bacterial density (*y*) of the high-temperature adapted clones (*l*), measured in different days (*d*) and with several replicates (*r*).

**Table S1. Statistical analyses summary of the relative fitnesses of the 114 high-temperature adapted clones at 20 °C, 37 °C (this study), and 42.2°**

| High-temperature adapted clones | Mean fitness at 20 °C ± SE[a] | | ±95% CI[b] | Significance (*P* value)[c] | Mean fitness at 37 °C ± SE[a] | | ±95% CI[b] | Significance (*P* value)[c] | Mean fitness at 42.2 °C ± SE[a] (1) | | ±95% CI[b] | Significance (*P* value)[c] |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | 0.853 | 0.013 | 0.058 | 0.008** | 0.998 | 0.016 | 0.067 | 0.924 | 1.409 | 0.024 | 0.063 | <0.001*** |
| 2 | 0.986 | 0.035 | 0.151 | 0.723 | 0.989 | 0.031 | 0.134 | 0.753 | 1.484 | 0.062 | 0.160 | 0.001** |
| 3 | 0.981 | 0.019 | 0.050 | 0.370 | 0.922 | 0.013 | 0.057 | 0.028* | 1.257 | 0.065 | 0.167 | 0.011* |
| 4 | 0.936 | 0.023 | 0.058 | 0.038* | 1.004 | 0.014 | 0.062 | 0.820 | 1.327 | 0.079 | 0.202 | 0.009** |
| 5 | 1.023 | 0.024 | 0.062 | 0.386 | 0.958 | 0.032 | 0.139 | 0.326 | 1.559 | 0.109 | 0.279 | 0.004** |
| 7 | 0.980 | 0.028 | 0.120 | 0.553 | 0.874 | 0.029 | 0.123 | 0.048* | 1.529 | 0.054 | 0.139 | <0.001*** |
| 8 | 0.958 | 0.013 | 0.033 | 0.023* | 0.935 | 0.030 | 0.130 | 0.164 | 1.525 | 0.032 | 0.082 | <0.001*** |
| 9 | 0.834 | 0.013 | 0.032 | 0.000*** | 0.970 | 0.036 | 0.153 | 0.493 | 1.472 | 0.051 | 0.130 | <0.001*** |
| 10 | 1.011 | 0.009 | 0.040 | 0.355 | 1.019 | 0.033 | 0.085 | 0.598 | 1.667 | 0.072 | 0.186 | <0.001*** |
| 11 | 0.866 | 0.029 | 0.123 | 0.043* | 0.826 | 0.025 | 0.108 | 0.020* | 1.244 | 0.032 | 0.083 | 0.001** |
| 12 | 0.882 | 0.028 | 0.121 | 0.053. | 0.960 | 0.019 | 0.048 | 0.084. | 1.323 | 0.048 | 0.124 | 0.001** |
| 13 | 0.926 | 0.011 | 0.046 | 0.020* | 0.986 | 0.018 | 0.076 | 0.504 | 1.566 | 0.078 | 0.200 | 0.001** |
| 14 | 0.897 | 0.003 | 0.014 | 0.001** | 1.006 | 0.023 | 0.060 | 0.819 | 1.603 | 0.090 | 0.231 | 0.001** |
| 15 | 0.973 | 0.021 | 0.089 | 0.321 | 0.990 | 0.045 | 0.192 | 0.841 | 1.499 | 0.070 | 0.180 | 0.001** |
| 16 | 0.888 | 0.016 | 0.068 | 0.019* | 0.948 | 0.028 | 0.120 | 0.201 | 1.272 | 0.070 | 0.180 | 0.012* |
| 17 | 0.973 | 0.025 | 0.106 | 0.381 | 0.944 | 0.031 | 0.132 | 0.207 | 1.460 | 0.044 | 0.113 | <0.001*** |
| 18 | 0.698 | 0.065 | 0.167 | 0.043* | 0.889 | 0.026 | 0.110 | 0.049* | 1.561 | 0.104 | 0.267 | 0.003** |
| 20 | 0.861 | 0.028 | 0.120 | 0.038* | 0.995 | 0.023 | 0.097 | 0.845 | 1.278 | 0.053 | 0.137 | 0.003** |
| 21 | 0.967 | 0.023 | 0.097 | 0.286 | 1.019 | 0.016 | 0.040 | 0.272 | 1.478 | 0.118 | 0.303 | 0.010* |
| 22 | 0.867 | 0.021 | 0.053 | 0.001** | 0.962 | 0.003 | 0.014 | 0.008** | 1.325 | 0.042 | 0.107 | 0.001** |
| 23 | 0.996 | 0.018 | 0.076 | 0.837 | 1.027 | 0.030 | 0.131 | 0.464 | 1.129 | 0.035 | 0.089 | 0.014* |
| 24 | 0.900 | 0.025 | 0.065 | 0.011* | 1.003 | 0.024 | 0.103 | 0.913 | 1.570 | 0.067 | 0.172 | <0.001*** |
| 25 | 0.897 | 0.011 | 0.027 | 0.000*** | 0.946 | 0.035 | 0.151 | 0.261 | 1.184 | 0.038 | 0.098 | 0.005** |
| 26 | 1.006 | 0.025 | 0.106 | 0.842 | 0.939 | 0.011 | 0.048 | 0.031* | 1.503 | 0.037 | 0.102 | <0.001*** |
| 27 | 0.900 | 0.014 | 0.036 | 0.001** | 0.966 | 0.007 | 0.030 | 0.039* | 1.243 | 0.058 | 0.150 | 0.009** |
| 28 | 0.790 | 0.009 | 0.022 | 0.000*** | 1.010 | 0.044 | 0.188 | 0.846 | 1.503 | 0.083 | 0.214 | 0.002** |
| 31 | 0.742 | 0.037 | 0.161 | 0.020* | 0.997 | 0.041 | 0.175 | 0.947 | 1.330 | 0.049 | 0.126 | 0.001** |
| 32 | 0.948 | 0.016 | 0.068 | 0.080. | 1.034 | 0.021 | 0.054 | 0.169 | 1.290 | 0.042 | 0.109 | 0.001** |
| 33 | 0.646 | 0.020 | 0.084 | 0.003** | 1.014 | 0.009 | 0.038 | 0.247 | 1.402 | 0.036 | 0.092 | <0.001*** |
| 34 | 0.978 | 0.029 | 0.124 | 0.521 | 1.013 | 0.047 | 0.202 | 0.812 | 1.510 | 0.059 | 0.152 | <0.001*** |
| 35 | 0.942 | 0.010 | 0.044 | 0.030* | 1.041 | 0.019 | 0.049 | 0.084. | 1.473 | 0.062 | 0.159 | 0.001** |
| 38 | 0.964 | 0.041 | 0.176 | 0.476 | 0.968 | 0.022 | 0.096 | 0.292 | 1.483 | 0.054 | 0.140 | <0.001*** |
| 39 | 0.956 | 0.015 | 0.066 | 0.104 | 0.968 | 0.018 | 0.076 | 0.210 | 1.437 | 0.068 | 0.174 | 0.001** |
| 40 | 0.706 | 0.050 | 0.216 | 0.028* | 0.942 | 0.005 | 0.020 | 0.006** | 1.368 | 0.037 | 0.095 | <0.001*** |
| 41 | 0.897 | 0.012 | 0.051 | 0.013* | 0.959 | 0.008 | 0.032 | 0.032* | 1.306 | 0.061 | 0.157 | 0.004** |
| 42 | 0.834 | 0.038 | 0.164 | 0.049* | 0.964 | 0.036 | 0.156 | 0.422 | 1.561 | 0.053 | 0.137 | <0.001*** |
| 43 | 0.865 | 0.018 | 0.047 | 0.001** | 0.970 | 0.041 | 0.176 | 0.544 | 1.496 | 0.048 | 0.124 | <0.001*** |
| 44 | 0.932 | 0.052 | 0.224 | 0.320 | 1.028 | 0.019 | 0.048 | 0.192 | 1.516 | 0.078 | 0.201 | 0.001** |
| 45 | 0.942 | 0.025 | 0.109 | 0.149 | 0.993 | 0.022 | 0.095 | 0.793 | 1.293 | 0.061 | 0.156 | 0.005** |
| 46 | 0.941 | 0.034 | 0.145 | 0.222 | 1.030 | 0.013 | 0.035 | 0.077. | 1.473 | 0.063 | 0.161 | 0.001** |
| 47 | 0.875 | 0.025 | 0.106 | 0.036* | 1.065 | 0.080 | 0.346 | 0.501 | 1.546 | 0.077 | 0.197 | 0.001** |
| 48 | 0.929 | 0.014 | 0.061 | 0.038* | 0.967 | 0.020 | 0.088 | 0.253 | 1.298 | 0.065 | 0.180 | 0.010* |
| 51 | 0.905 | 0.020 | 0.086 | 0.042* | 1.004 | 0.013 | 0.056 | 0.798 | 1.349 | 0.078 | 0.200 | 0.006** |
| 52 | 1.009 | 0.061 | 0.265 | 0.901 | 0.972 | 0.027 | 0.115 | 0.411 | 1.415 | 0.053 | 0.136 | 0.001** |
| 53 | 0.911 | 0.029 | 0.125 | 0.093. | 0.982 | 0.028 | 0.122 | 0.581 | 1.096 | 0.043 | 0.110 | 0.074. |
| 54 | 0.916 | 0.012 | 0.050 | 0.018* | 0.995 | 0.035 | 0.149 | 0.904 | 1.384 | 0.079 | 0.204 | 0.005** |
| 55 | 0.792 | 0.034 | 0.146 | 0.026* | 0.896 | 0.023 | 0.100 | 0.046* | 1.390 | 0.091 | 0.235 | 0.008** |
| 56 | 1.035 | 0.018 | 0.047 | 0.117 | 0.940 | 0.015 | 0.039 | 0.011* | 1.319 | 0.070 | 0.179 | 0.006** |
| 57 | 0.779 | 0.050 | 0.129 | 0.007** | 0.939 | 0.010 | 0.026 | 0.002** | 1.279 | 0.043 | 0.110 | 0.001** |
| 58 | 0.932 | 0.008 | 0.019 | 0.012* | 0.832 | 0.020 | 0.087 | 0.014* | 1.419 | 0.092 | 0.235 | 0.006** |
| 59 | 0.845 | 0.024 | 0.102 | 0.023* | 0.954 | 0.024 | 0.102 | 0.189 | 1.383 | 0.066 | 0.170 | 0.002** |
| 60 | 0.993 | 0.017 | 0.072 | 0.723 | 1.016 | 0.021 | 0.053 | 0.487 | 1.277 | 0.043 | 0.110 | 0.001** |
| 61 | 0.835 | 0.030 | 0.130 | 0.032* | 0.998 | 0.026 | 0.112 | 0.952 | 1.536 | 0.107 | 0.276 | 0.004** |
| 64 | 0.870 | 0.004 | 0.018 | 0.001** | 0.906 | 0.008 | 0.022 | 0.000*** | 1.495 | 0.073 | 0.187 | 0.001** |
| 65 | 0.914 | 0.004 | 0.018 | 0.002** | 1.030 | 0.049 | 0.213 | 0.604 | 1.405 | 0.052 | 0.134 | 0.001** |
| 66 | 1.033 | 0.011 | 0.028 | 0.029* | 0.941 | 0.009 | 0.040 | 0.024* | 1.430 | 0.059 | 0.151 | 0.001** |
| 67 | 0.926 | 0.025 | 0.106 | 0.095. | 0.969 | 0.006 | 0.027 | 0.037* | 1.378 | 0.032 | 0.082 | <0.001*** |
| 68 | 0.892 | 0.013 | 0.055 | 0.014* | 0.882 | 0.022 | 0.097 | 0.034* | 1.283 | 0.060 | 0.155 | 0.005** |
| 69 | 0.907 | 0.032 | 0.138 | 0.102 | 1.041 | 0.032 | 0.137 | 0.322 | 1.541 | 0.047 | 0.121 | <0.001*** |
| 70 | 1.029 | 0.015 | 0.063 | 0.183 | 0.992 | 0.025 | 0.064 | 0.752 | 1.308 | 0.035 | 0.091 | <0.001*** |

**Table S1. Cont.**

| High-temperature adapted clones | Mean fitness at 20 °C ± SE[a] | | ±95% CI[b] | Significance (*P* value)[c] | Mean fitness at 37 °C ± SE[a] | | ±95% CI[b] | Significance (*P* value)[c] | Mean fitness at 42.2 °C ± SE[a] (1) | | ±95% CI[b] | Significance (*P* value)[c] |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 71 | 0.833 | 0.023 | 0.099 | 0.018* | 0.900 | 0.029 | 0.073 | 0.017* | 1.270 | 0.060 | 0.154 | 0.006** |
| 72 | 0.998 | 0.046 | 0.196 | 0.973 | 0.992 | 0.021 | 0.090 | 0.736 | 1.463 | 0.095 | 0.244 | 0.005** |
| 73 | 1.031 | 0.016 | 0.069 | 0.192 | 1.028 | 0.019 | 0.084 | 0.285 | 1.419 | 0.062 | 0.160 | 0.001** |
| 74 | 0.839 | 0.009 | 0.037 | 0.003** | 0.857 | 0.026 | 0.110 | 0.030* | 1.314 | 0.041 | 0.114 | 0.002** |
| 75 | 0.881 | 0.012 | 0.053 | 0.011* | 1.085 | 0.025 | 0.064 | 0.019* | 1.357 | 0.062 | 0.158 | 0.002** |
| 76 | 0.703 | 0.022 | 0.095 | 0.005** | 0.976 | 0.005 | 0.020 | 0.034* | 1.377 | 0.035 | 0.089 | <0.001*** |
| 77 | 0.912 | 0.022 | 0.094 | 0.057. | 0.930 | 0.004 | 0.016 | 0.003** | 1.430 | 0.096 | 0.248 | 0.007** |
| 78 | 1.004 | 0.013 | 0.056 | 0.790 | 1.033 | 0.062 | 0.269 | 0.653 | 1.570 | 0.096 | 0.247 | 0.002** |
| 79 | 0.953 | 0.014 | 0.062 | 0.081. | 0.977 | 0.018 | 0.078 | 0.326 | 1.238 | 0.048 | 0.132 | 0.008** |
| 80 | 0.955 | 0.030 | 0.078 | 0.196 | 1.003 | 0.038 | 0.165 | 0.953 | 1.270 | 0.079 | 0.203 | 0.019* |
| 81 | 0.864 | 0.025 | 0.064 | 0.003** | 1.016 | 0.015 | 0.037 | 0.321 | 1.480 | 0.078 | 0.201 | 0.002** |
| 82 | 0.876 | 0.019 | 0.050 | 0.001** | 0.939 | 0.017 | 0.043 | 0.015* | 1.498 | 0.053 | 0.137 | <0.001*** |
| 83 | 0.995 | 0.030 | 0.128 | 0.881 | 0.970 | 0.026 | 0.112 | 0.374 | 1.178 | 0.036 | 0.092 | 0.004** |
| 84 | 0.978 | 0.019 | 0.080 | 0.353 | 0.999 | 0.017 | 0.044 | 0.971 | 1.180 | 0.070 | 0.181 | 0.051. |
| 85 | 0.848 | 0.020 | 0.085 | 0.017* | 0.996 | 0.013 | 0.034 | 0.758 | 1.662 | 0.087 | 0.222 | 0.001** |
| 86 | 0.866 | 0.014 | 0.061 | 0.011* | 0.952 | 0.019 | 0.050 | 0.055. | 1.310 | 0.077 | 0.198 | 0.010* |
| 87 | 1.010 | 0.032 | 0.139 | 0.781 | 1.015 | 0.032 | 0.139 | 0.689 | 1.703 | 0.137 | 0.351 | 0.004** |
| 89 | 0.941 | 0.044 | 0.189 | 0.313 | 0.975 | 0.041 | 0.177 | 0.600 | 1.387 | 0.046 | 0.117 | <0.001*** |
| 91 | 0.784 | 0.022 | 0.095 | 0.010* | 0.990 | 0.023 | 0.098 | 0.695 | 1.472 | 0.057 | 0.146 | <0.001*** |
| 92 | 0.825 | 0.009 | 0.040 | 0.003** | 0.918 | 0.016 | 0.068 | 0.035* | 1.446 | 0.093 | 0.239 | 0.005** |
| 93 | 0.907 | 0.011 | 0.049 | 0.015* | 1.010 | 0.019 | 0.081 | 0.650 | 1.396 | 0.040 | 0.103 | <0.001*** |
| 94 | 0.968 | 0.001 | 0.005 | 0.001** | 0.941 | 0.007 | 0.028 | 0.012* | 1.767 | 0.134 | 0.344 | 0.002** |
| 95 | 0.903 | 0.001 | 0.003 | 0.000*** | 0.921 | 0.013 | 0.034 | 0.002** | 1.488 | 0.109 | 0.280 | 0.007** |
| 96 | 0.934 | 0.048 | 0.124 | 0.302 | 0.990 | 0.018 | 0.077 | 0.641 | 1.439 | 0.105 | 0.270 | 0.009** |
| 97 | 0.926 | 0.105 | 0.269 | 0.512 | 0.978 | 0.019 | 0.049 | 0.310 | 1.277 | 0.096 | 0.247 | 0.034* |
| 101 | 0.976 | 0.018 | 0.047 | 0.248 | 1.014 | 0.016 | 0.041 | 0.438 | 1.404 | 0.031 | 0.079 | <0.001*** |
| 105 | 0.939 | 0.020 | 0.088 | 0.095. | 1.025 | 0.020 | 0.087 | 0.338 | 1.397 | 0.038 | 0.097 | <0.001*** |
| 106 | 0.928 | 0.008 | 0.035 | 0.012* | 1.016 | 0.031 | 0.079 | 0.620 | 1.383 | 0.100 | 0.258 | 0.012* |
| 107 | Could not grow at 20 °C | | | | 0.982 | 0.011 | 0.028 | 0.168 | 1.635 | 0.061 | 0.157 | <0.001*** |
| 108 | 0.870 | 0.003 | 0.014 | 0.001** | 0.989 | 0.020 | 0.085 | 0.642 | 1.671 | 0.112 | 0.289 | 0.002** |
| 110 | 0.913 | 0.028 | 0.072 | 0.089. | 1.007 | 0.006 | 0.024 | 0.361 | 1.469 | 0.073 | 0.187 | 0.001** |
| 112 | 0.906 | 0.004 | 0.018 | 0.002** | 0.992 | 0.019 | 0.048 | 0.693 | 1.230 | 0.085 | 0.218 | 0.042* |
| 114 | 0.830 | 0.012 | 0.052 | 0.005** | 0.967 | 0.005 | 0.022 | 0.023* | 1.217 | 0.063 | 0.162 | 0.018* |
| 118 | 0.849 | 0.022 | 0.056 | 0.001** | 0.935 | 0.004 | 0.017 | 0.004** | 1.612 | 0.039 | 0.101 | <0.001*** |
| 119 | 0.716 | 0.015 | 0.038 | 0.003** | 0.953 | 0.009 | 0.038 | 0.033* | 1.626 | 0.098 | 0.253 | 0.001** |
| 120 | 0.807 | 0.020 | 0.086 | 0.011* | 0.954 | 0.015 | 0.039 | 0.029* | 1.300 | 0.055 | 0.153 | 0.005** |
| 122 | 0.908 | 0.015 | 0.066 | 0.027* | 0.975 | 0.044 | 0.189 | 0.626 | 1.381 | 0.033 | 0.084 | <0.001*** |
| 124 | 0.935 | 0.017 | 0.044 | 0.012* | 0.913 | 0.015 | 0.066 | 0.030* | 1.427 | 0.048 | 0.122 | <0.001*** |
| 126 | 0.998 | 0.017 | 0.043 | 0.929 | 0.970 | 0.025 | 0.108 | 0.358 | 1.544 | 0.061 | 0.157 | <0.001*** |
| 127 | 0.890 | 0.025 | 0.063 | 0.006** | 1.003 | 0.012 | 0.031 | 0.822 | 1.387 | 0.047 | 0.120 | <0.001*** |
| 130 | 0.999 | 0.011 | 0.028 | 0.935 | 0.990 | 0.010 | 0.041 | 0.392 | 1.284 | 0.068 | 0.188 | 0.014* |
| 131 | 0.968 | 0.026 | 0.114 | 0.348 | 0.948 | 0.012 | 0.050 | 0.047* | 1.345 | 0.036 | 0.093 | <0.001*** |
| 132 | 1.009 | 0.015 | 0.066 | 0.601 | 0.980 | 0.053 | 0.227 | 0.737 | 1.432 | 0.082 | 0.212 | 0.003** |
| 133 | 0.684 | 0.012 | 0.053 | 0.002** | 0.919 | 0.004 | 0.015 | 0.002** | 1.379 | 0.070 | 0.180 | 0.003** |
| 134 | 1.032 | 0.017 | 0.044 | 0.118 | 0.985 | 0.017 | 0.044 | 0.408 | 1.380 | 0.053 | 0.136 | 0.001** |
| 135 | 0.947 | 0.018 | 0.046 | 0.031* | 0.993 | 0.017 | 0.074 | 0.715 | 1.473 | 0.056 | 0.143 | <0.001*** |
| 136 | 0.911 | 0.007 | 0.018 | 0.000*** | 0.886 | 0.000 | 0.002 | 0.000*** | 1.295 | 0.028 | 0.072 | <0.001*** |
| 137 | 0.932 | 0.006 | 0.025 | 0.008** | 0.927 | 0.008 | 0.036 | 0.013* | 1.349 | 0.041 | 0.104 | <0.001*** |
| 138 | 0.968 | 0.021 | 0.088 | 0.261 | 1.011 | 0.017 | 0.043 | 0.558 | 1.433 | 0.081 | 0.209 | 0.003** |
| 139 | 0.877 | 0.015 | 0.039 | 0.000*** | 0.966 | 0.024 | 0.104 | 0.299 | 1.375 | 0.053 | 0.135 | 0.001** |
| 140 | 0.981 | 0.024 | 0.104 | 0.516 | 1.024 | 0.031 | 0.134 | 0.522 | 1.648 | 0.039 | 0.101 | <0.001*** |
| 141 | 0.991 | 0.025 | 0.064 | 0.736 | 0.956 | 0.009 | 0.038 | 0.038* | 1.460 | 0.055 | 0.141 | <0.001*** |
| 142 | 0.885 | 0.015 | 0.038 | 0.001** | 0.959 | 0.033 | 0.142 | 0.336 | 1.609 | 0.067 | 0.172 | <0.001*** |
| 143 | 0.927 | 0.017 | 0.043 | 0.007** | 0.967 | 0.029 | 0.127 | 0.375 | 1.540 | 0.081 | 0.208 | 0.001** |

[a]Mean relative fitness and SEM.
[b]95% confidence interval (CI).
[c]The null hypothesis is that mean fitness relative to the ancestor equals 1; probabilities calculated using a two-tailed *t* test. The asterisks represent significant deviation from the null hypothesis that mean fitness equals 1.0, with a single asterisk denoting significance at $0.01 < P < 0.05$, double asterisks denoting significance at $0.001 < P < 0.01$, and triple asterisks denoting significance at $P < 0.001$ and a dot denoting $0.05 < P < 0.1$.

1. Tenaillon O, et al. (2012) The molecular diversity of adaptive convergence. *Science* 335():457–461.

**Table S2. Statistical analyses summary of the absolute fitnesses of the ancestor and the 114 high-temperature adapted clones at 17 °C, 18 °C, 19 °C, and 45 °C**

| High-temperature adapted clones | Mean absolute fitness at 17 °C[a] | 95% CI (upper limit)[b] | Less than 0?[c] | Mean absolute fitness at 18 °C[a] | 95% CI (upper limit)[b] | Less than 0?[c] | Mean absolute fitness at 19 °C[a] | 95% CI (upper limit)[b] | Less than 0?[c] | Mean absolute fitness at 45 °C[a] | 95% CI (upper limit)[b] | Less than 0?[c] |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | −1.015 | −0.751 | YES | −0.415 | −0.352 | YES | −0.134 | −0.006 | YES | −0.092 | 0.095 | NO |
| 2 | −0.301 | −0.059 | YES | 0.057 | 0.134 | NO | 0.018 | 0.091 | NO | −0.916 | −0.312 | YES |
| 3 | −0.896 | −0.684 | YES | −0.024 | 0.048 | NO | 0.022 | 0.121 | NO | 0.015 | 0.062 | NO |
| 4 | −1.300 | −1.084 | YES | −0.543 | −0.405 | YES | −0.219 | −0.067 | YES | −0.038 | 0.017 | NO |
| 5 | −0.931 | −0.818 | YES | −0.089 | 0.095 | NO | 0.115 | 0.216 | NO | −0.036 | 0.041 | NO |
| 7 | −0.480 | −0.303 | YES | −0.034 | 0.082 | NO | 0.073 | 0.126 | NO | 0.016 | 0.064 | NO |
| 8 | −0.867 | −0.551 | YES | −0.260 | −0.017 | YES | 0.096 | 0.213 | NO | −0.062 | 0.021 | NO |
| 9 | −1.627 | −1.180 | YES | −0.791 | −0.696 | YES | −0.285 | −0.049 | YES | −0.032 | 0.035 | NO |
| 10 | −0.893 | −0.572 | YES | −0.221 | −0.054 | YES | 0.034 | 0.100 | NO | −0.109 | 0.024 | NO |
| 11 | −1.254 | −0.530 | YES | −0.852 | −0.325 | YES | −0.688 | −0.549 | YES | −0.003 | 0.055 | NO |
| 12 | −1.099 | −0.456 | YES | −0.550 | −0.314 | YES | −0.076 | 0.130 | NO | −0.061 | 0.033 | NO |
| 13 | −0.752 | −0.507 | YES | 0.056 | 0.164 | NO | 0.033 | 0.089 | NO | −0.032 | 0.042 | NO |
| 14 | −1.025 | −0.774 | YES | −0.314 | −0.177 | YES | −0.114 | 0.061 | NO | 0.022 | 0.080 | NO |
| 15 | −0.458 | −0.171 | YES | 0.033 | 0.088 | NO | 0.039 | 0.131 | NO | 0.052 | 0.095 | NO |
| 16 | −0.952 | −0.770 | YES | −0.393 | −0.294 | YES | −0.072 | 0.014 | NO | −0.029 | 0.037 | NO |
| 17 | −0.658 | −0.485 | YES | −0.104 | −0.020 | YES | 0.055 | 0.130 | NO | 0.004 | 0.064 | NO |
| 18 | −0.937 | −0.492 | YES | −0.746 | −0.174 | YES | −0.316 | −0.068 | YES | 0.035 | 0.076 | NO |
| 20 | −1.221 | −0.775 | YES | −0.609 | −0.447 | YES | −0.072 | 0.105 | NO | −0.004 | 0.065 | NO |
| 21 | −0.964 | −0.705 | YES | −0.059 | 0.027 | NO | 0.008 | 0.110 | NO | 0.018 | 0.080 | NO |
| 22 | −1.262 | −1.024 | YES | −0.586 | −0.485 | YES | 0.043 | 0.132 | NO | −0.025 | 0.037 | NO |
| 23 | −0.434 | −0.190 | YES | 0.048 | 0.105 | NO | −0.063 | −0.002 | YES[d] | −0.011 | 0.052 | NO |
| 24 | −1.250 | −0.863 | YES | −0.478 | −0.287 | YES | −0.300 | −0.120 | YES | −0.069 | 0.006 | NO |
| 25 | −1.000 | −0.790 | YES | −0.388 | −0.258 | YES | −0.057 | 0.008 | NO | −0.081 | 0.016 | NO |
| 26 | −1.155 | −1.033 | YES | 0.079 | 0.188 | NO | 0.006 | 0.074 | NO | 0.029 | 0.115 | NO |
| 27 | −1.236 | −0.981 | YES | −0.272 | −0.188 | YES | −0.052 | 0.051 | NO | 0.028 | 0.057 | NO |
| 28 | −1.245 | −0.666 | YES | −0.306 | −0.231 | YES | −0.036 | 0.024 | NO | −0.012 | 0.052 | NO |
| 31 | −1.200 | −0.893 | YES | −1.003 | −0.725 | YES | −0.334 | −0.127 | YES | −0.016 | 0.037 | NO |
| 32 | −1.343 | −0.795 | YES | 0.013 | 0.094 | NO | −0.037 | 0.021 | NO | −0.038 | 0.034 | NO |
| 33 | −1.847 | −1.259 | YES | −1.424 | −0.674 | YES | −0.216 | 0.220 | NO | −0.013 | 0.044 | NO |
| 34 | −1.071 | −0.907 | YES | −0.113 | −0.047 | YES | −0.082 | 0.020 | NO | 0.000 | 0.076 | NO |
| 35 | −1.077 | −0.741 | YES | −0.236 | −0.125 | YES | 0.023 | 0.173 | NO | −0.024 | 0.035 | NO |
| 38 | −0.991 | −0.827 | YES | 0.029 | 0.131 | NO | −0.016 | 0.062 | NO | −0.061 | 0.005 | NO |
| 39 | −0.825 | −0.686 | YES | −0.005 | 0.078 | NO | −0.042 | 0.024 | NO | −0.056 | 0.014 | NO |
| 40 | −1.395 | −1.192 | YES | −0.928 | −0.823 | YES | −0.276 | −0.060 | YES | −0.038 | 0.012 | NO |
| 41 | −1.078 | −0.746 | YES | −0.067 | 0.029 | NO | −0.026 | 0.057 | NO | −0.015 | 0.058 | NO |
| 42 | −0.948 | −0.680 | YES | 0.020 | 0.083 | NO | 0.043 | 0.115 | NO | −0.016 | 0.052 | NO |
| 43 | −1.071 | −0.753 | YES | −0.276 | −0.165 | YES | 0.066 | 0.146 | NO | 0.012 | 0.084 | NO |
| 44 | −0.724 | −0.522 | YES | 0.004 | 0.088 | NO | 0.027 | 0.131 | NO | 0.014 | 0.075 | NO |
| 45 | −1.151 | −0.913 | YES | −0.428 | −0.232 | YES | −0.034 | 0.118 | NO | −0.002 | 0.072 | NO |
| 46 | −1.165 | −0.925 | YES | −0.112 | 0.015 | NO | 0.037 | 0.103 | NO | 0.028 | 0.078 | NO |
| 47 | −1.691 | −1.274 | YES | −0.598 | −0.449 | YES | −0.105 | 0.077 | NO | 0.010 | 0.050 | NO |
| 48 | −0.704 | −0.456 | YES | −0.022 | 0.100 | NO | 0.050 | 0.159 | NO | −0.001 | 0.083 | NO |
| 51 | −0.815 | −0.583 | YES | 0.011 | 0.090 | NO | 0.049 | 0.146 | NO | 0.015 | 0.071 | NO |
| 52 | −0.867 | −0.613 | YES | 0.020 | 0.089 | NO | 0.027 | 0.110 | NO | 0.000 | 0.045 | NO |
| 53 | −1.124 | −0.966 | YES | −0.018 | 0.070 | NO | 0.069 | 0.176 | NO | −0.007 | 0.053 | NO |
| 54 | −0.621 | −0.392 | YES | 0.024 | 0.122 | NO | 0.085 | 0.129 | NO | −0.016 | 0.039 | NO |
| 55 | −1.944 | −1.444 | YES | −0.713 | −0.637 | YES | −0.231 | −0.070 | YES | −0.018 | 0.082 | NO |
| 56 | −1.274 | −0.956 | YES | −0.176 | −0.065 | YES | 0.070 | 0.125 | NO | 0.003 | 0.108 | NO |
| 57 | −1.984 | −1.181 | YES | −1.137 | −0.805 | YES | −0.248 | −0.011 | YES | −0.017 | 0.041 | NO |
| 58 | −1.509 | 0.613 | NO[d] | −1.205 | −0.582 | YES | −0.185 | 0.143 | NO | 0.021 | 0.088 | NO |
| 59 | −1.610 | −1.273 | YES | −1.048 | −0.924 | YES | −0.070 | 0.043 | NO | −0.027 | 0.006 | NO |
| 60 | −0.990 | −0.676 | YES | 0.043 | 0.134 | NO | 0.047 | 0.149 | NO | −0.074 | −0.017 | YES |
| 61 | −1.409 | −1.165 | YES | −0.378 | −0.211 | YES | −0.084 | 0.102 | NO | −0.026 | 0.030 | NO |
| 64 | −1.396 | −1.151 | YES | −0.353 | −0.193 | YES | 0.085 | 0.179 | NO | −0.042 | 0.006 | NO |
| 65 | −1.425 | −1.057 | YES | −0.079 | 0.030 | NO | −0.005 | 0.068 | NO | −0.046 | 0.020 | NO |
| 66 | −0.904 | −0.545 | YES | 0.120 | 0.223 | NO | 0.053 | 0.111 | NO | −0.017 | 0.030 | NO |
| 67 | −1.369 | −0.894 | YES | −0.617 | −0.453 | YES | 0.005 | 0.109 | NO | −0.054 | 0.017 | NO |
| 68 | −1.437 | −0.679 | YES | −0.576 | −0.427 | YES | −0.135 | 0.023 | NO | −0.042 | 0.078 | NO |
| 69 | −1.123 | −0.894 | YES | −0.490 | −0.348 | YES | 0.003 | 0.079 | NO | 0.012 | 0.078 | NO |

**Table S2. Cont.**

| High-temperature adapted clones | Mean absolute fitness at 17 °C[a] | 95% CI (upper limit)[b] | Less than 0?[c] | Mean absolute fitness at 18 °C[a] | 95% CI (upper limit)[b] | Less than 0?[c] | Mean absolute fitness at 19 °C[a] | 95% CI (upper limit)[b] | Less than 0?[c] | Mean absolute fitness at 45 °C[a] | 95% CI (upper limit)[b] | Less than 0?[c] |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 70 | −0.885 | −0.670 | YES | 0.093 | 0.211 | NO | 0.054 | 0.169 | NO | −0.001 | 0.031 | NO |
| 71 | −1.313 | −0.828 | YES | −1.070 | −0.845 | YES | −0.166 | 0.059 | NO | −0.016 | 0.038 | NO |
| 72 | −1.352 | −1.068 | YES | −0.148 | 0.000 | YES | −0.060 | 0.098 | NO | −0.010 | 0.038 | NO |
| 73 | −0.648 | −0.367 | YES | −0.091 | 0.034 | NO | 0.092 | 0.166 | NO | −0.048 | 0.019 | NO |
| 74 | −1.193 | −0.245 | YES | −0.677 | −0.419 | YES | −0.129 | 0.063 | NO | 0.041 | 0.093 | NO |
| 75 | −1.871 | −1.511 | YES | −0.808 | −0.426 | YES | −0.093 | 0.097 | NO | −0.006 | 0.075 | NO |
| 76 | −1.305 | −1.026 | YES | −0.686 | −0.478 | YES | −0.606 | −0.447 | YES | −0.012 | 0.079 | NO |
| 77 | −1.199 | −0.947 | YES | −0.321 | −0.228 | YES | 0.021 | 0.114 | NO | −0.092 | −0.008 | YES |
| 78 | −0.634 | −0.546 | YES | 0.040 | 0.144 | NO | 0.039 | 0.116 | NO | −0.027 | 0.021 | NO |
| 79 | −1.189 | −1.035 | YES | −0.077 | 0.020 | NO | 0.077 | 0.156 | NO | −0.068 | 0.018 | NO |
| 80 | −1.058 | −0.818 | YES | 0.033 | 0.128 | NO | 0.012 | 0.081 | NO | −0.007 | 0.057 | NO |
| 81 | −0.885 | −0.714 | YES | 0.043 | 0.136 | NO | 0.049 | 0.104 | NO | −0.028 | 0.047 | NO |
| 82 | −0.674 | −0.416 | YES | 0.071 | 0.132 | NO | −0.003 | 0.079 | NO | −0.044 | 0.006 | NO |
| 83 | −1.128 | −0.716 | YES | −0.034 | 0.042 | NO | 0.043 | 0.108 | NO | −0.034 | 0.006 | NO |
| 84 | −0.913 | −0.540 | YES | −0.009 | 0.109 | NO | 0.062 | 0.139 | NO | −0.013 | 0.046 | NO |
| 85 | −1.602 | −0.979 | YES | −0.756 | −0.605 | YES | −0.206 | 0.039 | NO | −0.019 | 0.017 | NO |
| 86 | −1.587 | −1.280 | YES | −0.089 | 0.268 | NO | −0.041 | 0.061 | NO | −0.005 | 0.032 | NO |
| 87 | −0.607 | −0.510 | YES | 0.074 | 0.162 | NO | 0.044 | 0.111 | NO | −0.009 | 0.062 | NO |
| 89 | −0.995 | −0.802 | YES | −0.003 | 0.037 | NO | 0.040 | 0.122 | NO | −0.001 | 0.057 | NO |
| 91 | −1.584 | −1.189 | YES | −0.806 | −0.491 | YES | 0.020 | 0.094 | NO | −0.026 | 0.023 | NO |
| 92 | −1.943 | −1.370 | YES | −0.725 | −0.440 | YES | −0.353 | −0.101 | YES | −0.074 | 0.023 | NO |
| 93 | −1.079 | −0.735 | YES | −0.118 | 0.019 | NO | 0.048 | 0.094 | NO | −0.037 | 0.057 | NO |
| 94 | −0.844 | −0.570 | YES | 0.113 | 0.215 | NO | 0.070 | 0.141 | NO | 0.006 | 0.054 | NO |
| 95 | −0.981 | −0.822 | YES | 0.015 | 0.126 | NO | 0.022 | 0.081 | NO | −0.009 | 0.026 | NO |
| 96 | −0.747 | −0.573 | YES | 0.086 | 0.175 | NO | −0.015 | 0.084 | NO | −0.004 | 0.043 | NO |
| 97 | −1.270 | −0.675 | YES | −0.119 | 0.011 | NO | 0.032 | 0.075 | NO | −0.157 | 0.038 | NO |
| 101 | −0.838 | −0.539 | YES | 0.009 | 0.189 | NO | 0.097 | 0.182 | NO | 0.043 | 0.089 | NO |
| 105 | −0.591 | −0.343 | YES | 0.023 | 0.119 | NO | 0.036 | 0.094 | NO | −0.008 | 0.051 | NO |
| 106 | −1.258 | −0.974 | YES | 0.081 | 0.151 | NO | 0.092 | 0.159 | NO | −0.016 | 0.031 | NO |
| 107 | −0.956 | 1.094 | NO[d] | −0.690 | −0.334 | YES | −0.354 | −0.072 | YES | −0.032 | 0.023 | NO |
| 108 | −1.043 | −0.782 | YES | 0.080 | 0.162 | NO | 0.050 | 0.107 | NO | 0.026 | 0.068 | NO |
| 110 | −1.283 | −1.033 | YES | 0.136 | 0.380 | NO | 0.069 | 0.141 | NO | 0.028 | 0.066 | NO |
| 112 | −1.289 | −0.858 | YES | −0.024 | 0.045 | NO | −0.002 | 0.120 | NO | 0.039 | 0.074 | NO |
| 114 | −1.658 | −0.794 | YES | −0.808 | −0.680 | YES | −0.228 | 0.002 | NO | −0.380 | 0.058 | NO |
| 118 | −1.609 | −1.256 | YES | −0.372 | −0.249 | YES | −0.159 | 0.040 | NO | 0.044 | 0.088 | NO |
| 119 | −1.137 | −0.759 | YES | −0.298 | −0.124 | YES | −0.100 | 0.085 | NO | 0.005 | 0.076 | NO |
| 120 | −1.414 | −1.239 | YES | −0.223 | 0.005 | NO | −0.021 | 0.277 | NO | 0.041 | 0.083 | NO |
| 122 | −1.120 | −0.975 | YES | −0.664 | −0.430 | YES | 0.023 | 0.100 | NO | 0.023 | 0.099 | NO |
| 124 | −1.662 | −1.415 | YES | −0.536 | −0.360 | YES | −0.036 | 0.021 | NO | −0.008 | 0.084 | NO |
| 126 | −0.599 | −0.371 | YES | 0.012 | 0.037 | NO | 0.065 | 0.132 | NO | −0.052 | 0.042 | NO |
| 127 | −1.398 | −1.121 | YES | −0.257 | −0.083 | YES | −0.153 | 0.018 | NO | 0.014 | 0.097 | NO |
| 130 | −1.369 | −1.058 | YES | −0.454 | −0.303 | YES | −0.114 | −0.004 | YES | 0.060 | 0.157 | NO |
| 131 | −0.426 | −0.274 | YES | −0.035 | 0.031 | NO | 0.065 | 0.111 | NO | −0.137 | −0.066 | YES |
| 132 | −0.449 | −0.292 | YES | −0.010 | 0.099 | NO | 0.039 | 0.124 | NO | 0.200 | 0.500 | NO |
| 133 | −1.731 | −1.523 | YES | −0.834 | −0.669 | YES | −0.840 | −0.595 | YES | 0.049 | 0.157 | NO |
| 134 | −0.563 | −0.263 | YES | 0.004 | 0.089 | NO | 0.070 | 0.146 | NO | −0.319 | 0.172 | NO |
| 135 | −1.491 | −1.251 | YES | −0.387 | −0.234 | YES | −0.107 | 0.098 | NO | 0.038 | 0.095 | NO |
| 136 | −1.548 | −1.160 | YES | −0.364 | −0.286 | YES | −0.185 | 0.062 | NO | 0.024 | 0.072 | NO |
| 137 | −1.172 | −0.933 | YES | −0.111 | 0.028 | NO | 0.046 | 0.156 | NO | −0.015 | 0.024 | NO |
| 138 | −0.986 | −0.700 | YES | −0.061 | 0.047 | NO | 0.043 | 0.179 | NO | −0.033 | 0.033 | NO |
| 139 | −1.074 | −0.803 | YES | 0.018 | 0.118 | NO | 0.032 | 0.101 | NO | 0.016 | 0.054 | NO |
| 140 | −0.939 | −0.657 | YES | 0.020 | 0.088 | NO | 0.070 | 0.148 | NO | 0.014 | 0.063 | NO |
| 141 | −0.801 | −0.463 | YES | −0.005 | 0.068 | NO | 0.105 | 0.197 | NO | 0.000 | 0.072 | NO |
| 142 | −1.078 | −0.784 | YES | 0.001 | 0.129 | NO | 0.106 | 0.181 | NO | −0.067 | 0.000 | NO |
| 143 | −0.508 | −0.174 | YES | −0.023 | 0.041 | NO | 0.062 | 0.122 | NO | −0.062 | −0.012 | YES |
| REL1206 | −0.413 | −0.264 | YES | 0.000 | 0.075 | NO | −0.004 | 0.070 | NO | −0.290 | 0.097 | NO |

[a]Mean absolute fitness (slope of the fitted linear regression) based on three replicates.
[b]One-sided confidence interval at 95% based on 3 replicates.
[c]The null hypothesis is that the mean absolute fitness equals 0. If the one-sided interval is equal to zero, the absolute fitness is not less than zero (NO); if the one-sided interval is less than zero the absolute fitness is less than zero (YES).
[d]The populations go extinct between day 1 and 2, resulting in a large variance and an underestimation of the mean absolute fitness.

**Table S3. Oligonucleotides and primers used in this study**

| | |
|---|---|
| | Oligos used for recombineering to create *rpoB* and *rho* mutants |
| *rpoB* I966S (T2897G) | 5′-CAG CAC GGA TAC GGC TGA ACA GAC CCG CTT CGA GGC TCT GCA GTT CTT CAG ACA GGT CTT TCT TCG CCT G-3′ |
| *rho* I15N (T44A) | 5′-CAG GTT TTC CAG CCC CAT ATT TTC GCC GAG AGT GTT CAG CTC AGA AAC CGG CGT ATT CTT TAA TTC GGT-3′ |
| *araA* D92G (A275G) | 5′-CAC ACC TTC TCC CCG GCC AAA ATG TGG ATC AAC GGC CTG ACC ATG CTC AAC AAA CCG TTG CTG CAA TTC C-3′ |
| | Primer pair used for PCR amplification of *rpoB* or *rho* genic region |
| Primers to amplify the *rpoB* region (368 bp) | |
| I966 Forward | 5′-TAA GGT TAC GCC GAA AGG TG-3′ |
| I966 Reverse | 5′-GTT TCT CTT CGT CGG TCA GG-3′ |
| Primers to amplify the *rho* region (373 bp) | |
| I15 Forward | 5′-GAC CGT AAA CAG GCA TGG AT-3′ |
| I15 Reverse | 5′-GTA TCA CCA GTG CGG AGG TT-3′ |