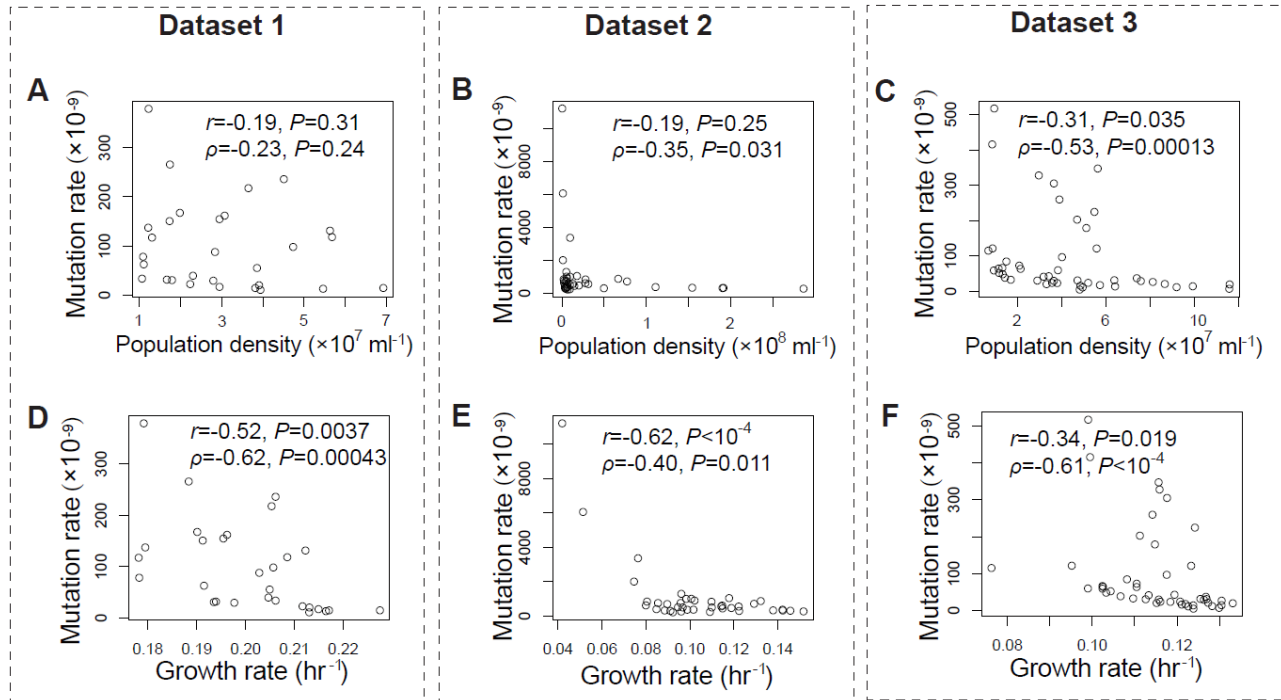


**Figure S1. Test of selection during MA in the seven environments. Related to Figure 2.** (A) Fractions of SNV mutations in genic and intergenic regions, respectively. (B) Fractions of nonsynonymous and synonymous mutations, respectively. Pink dashed lines represent expectations under no selection.



**Figure S2. Correlation between mutation rate and population density or growth rate. Related to Figure 3.** Dataset 1: rate of mutation to 5-Fluoro-orotic resistance in strain S288c; Dataset 2: rate of mutation to hygromycin B resistance in strain BY4742; Dataset 3: rate of mutation to hygromycin B resistance in strain Sigma1278b. (A), (B), and (C) show correlations between population density and mutation rate in these three datasets, whereas (D), (E), and (F) show correlations between growth rate and mutation rate in these three datasets. Spearman's  $\rho$  and Pearson's  $r$  are shown with corresponding  $P$  values. Data are from [1].

Environments	Recipes	Growth rate (hr <sup>-1</sup> ) before MA	Growth rate (hr <sup>-1</sup> ) after MA	Bottleneck interval	Number of bottlenecks	Number of cell divisions*
YPD	1% yeast extract, 2% peptone, and 2% dextrose	0.438	0.436	24 h	66	999.2
CuSO4	YPD with 6 mM CuSO4	0.445	0.441	24 h	64	982.4
YNB	0.17% yeast nitrogen base, 0.5% ammonium sulfate, and 2% dextrose <sup>#</sup>	0.364	0.356	36 h	56	1045.8
YPX	1% yeast extract, 2% peptone, and 2% xylose	0.330	0.324	36 h	62	1052.8
LiCl	YPD with 100 mM LiCl	0.266	0.254	48 h	58	1045.2
YPL	1% yeast extract, 2% peptone, and 2% lactose	0.253	0.247	48 h	57	987.2
NaCl	YPD with 1 M NaCl	0.251	0.236	48 h	62	1044.1

\* Total number of cell divisions is the number of bottlenecks × (number of cell divisions per bottleneck before MA + number of cell divisions per bottleneck after MA)/2.

<sup>#</sup> 2.5 ml 1 M NaOH is added to 1 L of medium for pH adjustment.

**Table S1. Information on the mutation accumulation experiments in seven environments. Related to Figure 1.**

Environments	SNV	Small indel	Segmental duplication/deletion	Chromosome gain/loss	Mitochondrial SNV	Mitochondrial indel	No. of cell divisions per line	No. of MA lines	Callable nuclear sites per line	Callable mitochondrial sites per line
YPD	112	11	1	1	12	25	999.2	24	24,055,032	85,773
CuSO4	125	21	0	1	10	18	982.4	23	24,055,032	85,779
YNB	198	17	0	3	5	6	1045.8	24	24,050,184	85,779
YPX	132	20	0	0	2	6	1052.8	24	24,042,864	85,779
LiCl	403	76	17	18	0	3	1045.2	23	24,058,360	82,553
YPL	166	19	0	2	2	7	987.2	24	24,020,272	85,735
NaCl	279	95	0	8	1	5	1044.1	24	24,035,698	85,779

**Table S2. Number of each type of mutations identified in each environment. Related to Figure 2.**

Sample	Genomic position	Ancestral allele	Mutant allele	Forward primer (5' to 3')	Reverse primer (5' to 3')	Result
NaCl-22	ref NC_001135 :83153	C	G	ACGCAAATGATGAGAAATAG	ATAATCGAATACCACATAGG	Confirmed
YPD-9	ref NC_001136 :149113	A	AAAATAAAT	AATCAAGTCCACGTCCAAGA	CAATGGCAACGATAACGAAG	Confirmed
YPX-14	ref NC_001136 :541173	T	A	CTTGGTAGCAGATATGGAAT	GTAATCAGAGTAGCTTTCA	Confirmed
YPL-20	ref NC_001139 :469242	A	ATTG	CTGGTAAATAGTGTGAAGA	TTCATTTAGGTCATTATCCG	Confirmed
LiCl-12	ref NC_001139 :549197	A	G	TTACATGCAGACTACATAGG	GCATGATCCAAATACGATTG	Confirmed
YPD-12	ref NC_001139 :789636	C	A	GAGCGTTACAAAGAATTAGG	GGTCGATATAGTAGGTAGCC	Confirmed
NaCl-6	ref NC_001141 :248934	AT	A	CTCGTCC TTCAGCCGTATAG	AAAAGTCATAGTCACCCACG	Confirmed
LiCl-9	ref NC_001142 :637260	C	A	GTCTTTAATCGTGCATTGGA	TTCCTTATACGTCAC TTATG	Confirmed
NaCl-12	ref NC_001143 :21369	C	A	AAATGGCAAATCATGTCCAC	TACGGCGAATAGTCTTCATT	Confirmed
CuSO <sub>4</sub> -10	ref NC_001143 :521153	C	G	CTAATTCCAATGCAGGTTCCG	CTAAGATCCGTGTGGTGGT	Confirmed
YNB-18	ref NC_001144 :213640	T	G	ATATTTCAATCTCTGCGTCG	TAGTTGATAGTAAGGGTGGT	Confirmed
YNB-19	ref NC_001144 :59747	T	TCTATATTGAAAAGTAAAC	TCCCTGTAGAGACTGAGGAG	GGACATAGTAATCAAGGCAC	Confirmed
YPL-8	ref NC_001145 :110268	G	T	GCTGACGTGCCATTAGAATC	CGTATTGACCCTTGCCTCTT	Confirmed
YPL-14	ref NC_001145 :65144	C	A	TCTGCACTTTGAAATGAACA	ATGATACTCGGTTCCGGTTGA	Confirmed
YPX-15	ref NC_001145 :888784	A	AT	GGTTCTGCCACTCTACAAAC	CAATCTAATATCTCCGCTGT	Confirmed
NaCl-16	ref NC_001147 :711615	G	GT	CAGTGGTAATGCAATAAAGG	GATGATTCCGACGATGACTA	Confirmed
CuSO <sub>4</sub> -10	ref NC_001148 :197730	ATATTATTATTGT	A	ATTCATAGGATACACAGCAT	GACTCACCTGTTCTGCTTTA	Confirmed
YPX-16	ref NC_001148 :435983	A	G	ATCACGAAGAGTCAATCAAT	ACAGTGTAAAGAAGATAACATA	Confirmed

**Table S3. Verification of mutations by Sanger sequencing. Related to Figure 2.**

Sample	SNV rate	small indel rate	chr gain/loss	seg dup/del	initial growth rate	end growth rate
Cu-1	5.16E-10	1.72E-10	0	0	0.445	0.430
Cu-3	2.51E-10	8.37E-11	0	0	0.449	0.449
Cu-4	3.29E-10	0	0	0	0.447	0.466
Cu-5	3.69E-10	0	0	0	0.398	0.417
Cu-6	4.07E-10	8.14E-11	0	0	0.461	0.462
Cu-7	3.23E-10	8.08E-11	0	0	0.468	0.462
Cu-8	3.29E-10	2.46E-10	0	0	0.458	0.457
Cu-9	9.82E-10	8.18E-11	0	0	0.458	0.461
Cu-10	5.93E-10	1.69E-10	0	0	0.468	0.420
Cu-11	4.29E-10	8.59E-11	0	0	0.452	0.424
Cu-12	1.67E-10	0	0.001001	0	0.447	0.454
Cu-13	4.22E-10	0	0	0	0.456	0.435
Cu-14	9.15E-10	0	0	0	0.465	0.439
Cu-15	2.59E-10	8.63E-11	0	0	0.437	0.435
Cu-16	5.14E-10	8.56E-11	0	0	0.442	0.437
Cu-17	7.47E-10	8.30E-11	0	0	0.461	0.445
Cu-18	3.42E-10	0	0	0	0.458	0.420
Cu-19	3.48E-10	1.74E-10	0	0	0.403	0.462
Cu-20	5.22E-10	1.74E-10	0	0	0.408	0.457
Cu-21	4.09E-10	0	0	0	0.468	0.451
Cu-22	7.09E-10	8.86E-11	0	0	0.403	0.445
Cu-23	3.51E-10	0	0	0	0.444	0.413
Cu-24	3.62E-10	9.06E-11	0	0	0.427	0.403
Li-1	1.75E-09	4.77E-10	0.000954	0	0.265	0.257
Li-2	1.61E-09	4.83E-10	0.000966	0	0.252	0.263
Li-3	1.91E-09	8.29E-11	0	0	0.248	0.252
Li-4	1.55E-09	1.63E-10	0	0	0.266	0.244
Li-5	1.66E-09	3.33E-10	0.001997	0.001997	0.259	0.240
Li-6	1.15E-09	4.10E-10	0	0	0.256	0.250
Li-7	1.62E-09	3.24E-10	0.001947	0	0.255	0.256
Li-9	1.13E-09	3.22E-10	0.001934	0	0.267	0.248
Li-10	7.75E-10	7.75E-11	0	0	0.264	0.271
Li-11	3.99E-10	7.98E-11	0.000957	0.001914	0.266	0.254
Li-12	8.03E-10	8.03E-11	0	0.000963	0.269	0.248
Li-13	1.09E-09	1.55E-10	0.001863	0	0.269	0.266
Li-14	1.47E-09	1.55E-10	0.000930	0	0.269	0.267
Li-15	1.71E-09	4.89E-10	0	0.000978	0.274	0.235
Li-16	1.82E-09	4.54E-10	0.001816	0	0.283	0.265
Li-17	1.30E-09	1.53E-10	0.000918	0	0.276	0.267
Li-18	2.10E-09	6.47E-10	0.000970	0.002910	0.261	0.252
Li-19	7.83E-10	7.83E-11	0	0	0.267	0.263
Li-20	7.80E-10	7.80E-11	0.001872	0	0.280	0.252
Li-21	1.55E-09	5.17E-10	0	0.006200	0.267	0.215
Li-22	1.45E-09	3.22E-10	0	0	0.271	0.244
Li-23	8.42E-10	7.66E-11	0	0	0.281	0.261
Li-24	3.00E-09	1.58E-10	0	0.001894	0.257	0.269
NaCl-1	7.47E-10	3.32E-10	0	0	0.246	0.222
NaCl-2	6.24E-10	2.34E-10	0	0	0.250	0.248
NaCl-3	5.60E-10	2.40E-10	0	0	0.241	0.244
NaCl-4	8.09E-10	8.09E-11	0	0	0.244	0.236
NaCl-5	9.62E-10	2.41E-10	0	0	0.252	0.232
NaCl-6	1.57E-09	4.96E-10	0.000992	0	0.231	0.239
NaCl-7	5.96E-10	5.11E-10	0	0	0.240	0.216
NaCl-8	1.02E-09	3.14E-10	0	0	0.258	0.237
NaCl-9	1.11E-09	1.58E-10	0.001898	0	0.253	0.238
NaCl-10	3.17E-10	0	0	0	0.236	0.255
NaCl-11	7.45E-10	1.65E-10	0	0	0.239	0.231
NaCl-12	1.14E-09	6.52E-10	0	0	0.250	0.226

NaCl-13	7.91E-10	2.37E-10	0	0	0.259	0.232
NaCl-14	1.25E-09	3.14E-10	0	0	0.248	0.247
NaCl-15	7.33E-10	3.26E-10	0	0	0.262	0.215
NaCl-16	1.05E-09	3.22E-10	0	0	0.254	0.228
NaCl-17	1.04E-09	4.01E-10	0.000962	0	0.260	0.225
NaCl-18	5.39E-10	2.31E-10	0	0	0.259	0.245
NaCl-19	7.27E-10	4.04E-10	0.000969	0	0.259	0.222
NaCl-20	1.62E-09	8.47E-10	0.000924	0	0.252	0.252
NaCl-21	7.24E-10	1.61E-10	0.000966	0	0.239	0.243
NaCl-22	1.45E-09	5.34E-10	0	0	0.259	0.250
NaCl-23	1.09E-09	2.33E-10	0.000933	0	0.263	0.236
NaCl-24	1.00E-09	1.54E-10	0	0	0.259	0.244
YNB-1	5.44E-10	0	0	0	0.370	0.367
YNB-2	4.71E-10	0	0	0	0.360	0.371
YNB-3	6.54E-10	0	0	0	0.345	0.355
YNB-4	3.96E-10	0	0	0	0.376	0.347
YNB-5	4.74E-10	0	0	0	0.363	0.363
YNB-6	9.66E-10	1.61E-10	0.000966	0	0.362	0.349
YNB-7	7.30E-10	8.11E-11	0	0	0.366	0.340
YNB-8	4.02E-10	8.03E-11	0	0	0.366	0.347
YNB-9	5.06E-10	0	0	0	0.350	0.329
YNB-10	4.85E-10	0	0	0	0.354	0.355
YNB-11	3.19E-10	0	0	0	0.352	0.366
YNB-12	1.13E-09	1.61E-10	0	0	0.355	0.355
YNB-13	2.34E-10	7.81E-11	0	0	0.370	0.363
YNB-14	6.08E-10	0	0.000913	0	0.374	0.380
YNB-15	7.44E-10	8.27E-11	0	0	0.351	0.342
YNB-16	7.80E-10	1.56E-10	0	0	0.367	0.367
YNB-17	7.34E-10	0	0	0	0.362	0.340
YNB-18	9.67E-10	1.61E-10	0	0	0.360	0.351
YNB-19	4.82E-10	2.41E-10	0	0	0.361	0.352
YNB-20	7.83E-10	0	0	0	0.382	0.350
YNB-21	1.24E-09	7.76E-11	0	0	0.376	0.363
YNB-22	7.13E-10	7.92E-11	0	0	0.375	0.348
YNB-23	6.34E-10	0	0.000951	0	0.363	0.360
YNB-24	7.77E-10	0	0	0	0.365	0.372
YPD-1	1.65E-10	0	0	0	0.469	0.413
YPD-2	4.14E-10	8.28E-11	0	0	0.444	0.437
YPD-3	4.12E-10	0	0	0	0.446	0.439
YPD-4	3.29E-10	0	0	0	0.458	0.430
YPD-5	2.43E-10	8.10E-11	0	0.000972	0.449	0.451
YPD-6	5.92E-10	0	0	0	0.401	0.461
YPD-7	6.69E-10	8.36E-11	0.001003	0	0.440	0.432
YPD-8	3.30E-10	2.47E-10	0	0	0.440	0.444
YPD-9	4.14E-10	8.29E-11	0	0	0.440	0.439
YPD-10	5.97E-10	0	0	0	0.446	0.408
YPD-11	2.51E-10	0	0	0	0.437	0.435
YPD-12	6.01E-10	0	0	0	0.433	0.417
YPD-13	1.74E-10	0	0	0	0.415	0.424
YPD-14	5.35E-10	0	0	0	0.391	0.427
YPD-15	4.31E-10	0	0	0	0.430	0.417
YPD-16	3.38E-10	1.69E-10	0	0	0.420	0.444
YPD-17	3.39E-10	0	0	0	0.444	0.417
YPD-18	1.70E-10	0	0	0	0.420	0.437
YPD-19	2.38E-10	0	0	0	0.454	0.465
YPD-20	4.89E-10	0	0	0	0.444	0.451
YPD-21	4.93E-10	0	0	0	0.430	0.458
YPD-22	5.76E-10	8.22E-11	0	0	0.470	0.417
YPD-23	2.47E-10	0	0	0	0.444	0.442
YPD-24	3.19E-10	7.97E-11	0	0	0.456	0.458
YPL-1	6.87E-10	0	0	0	0.254	0.238

YPL-2	4.23E-10	8.46E-11	0	0	0.257	0.242
YPL-3	5.12E-10	8.53E-11	0	0	0.255	0.240
YPL-4	1.08E-09	1.66E-10	0	0	0.260	0.249
YPL-5	8.31E-11	0	0	0	0.260	0.248
YPL-6	8.35E-10	0	0	0	0.269	0.237
YPL-7	6.80E-10	1.70E-10	0	0	0.248	0.249
YPL-8	1.02E-09	1.70E-10	0.001018	0	0.248	0.250
YPL-9	2.49E-10	8.30E-11	0	0	0.258	0.251
YPL-10	4.24E-10	8.47E-11	0	0	0.252	0.246
YPL-11	8.44E-11	0	0	0	0.265	0.236
YPL-12	3.25E-10	1.62E-10	0	0	0.264	0.256
YPL-13	4.95E-10	0	0	0	0.260	0.251
YPL-14	3.36E-10	1.68E-10	0	0	0.252	0.250
YPL-15	8.30E-10	8.30E-11	0	0	0.249	0.259
YPL-16	1.03E-09	0	0	0	0.240	0.249
YPL-17	6.81E-10	8.52E-11	0	0	0.247	0.248
YPL-18	5.08E-10	0	0	0	0.247	0.252
YPL-19	7.59E-10	8.44E-11	0.001013	0	0.255	0.245
YPL-20	5.10E-10	8.50E-11	0	0	0.254	0.243
YPL-21	2.50E-10	0	0	0	0.253	0.252
YPL-22	1.25E-09	0	0	0	0.238	0.237
YPL-23	7.77E-10	8.64E-11	0	0	0.239	0.249
YPL-24	2.57E-10	0	0	0	0.250	0.244
YPX-1	6.14E-10	7.68E-11	0	0	0.345	0.329
YPX-2	1.58E-10	1.58E-10	0	0	0.330	0.326
YPX-3	5.53E-10	0	0	0	0.335	0.320
YPX-4	3.12E-10	2.34E-10	0	0	0.331	0.333
YPX-5	3.99E-10	1.59E-10	0	0	0.332	0.317
YPX-6	2.46E-10	0	0	0	0.329	0.303
YPX-7	1.58E-10	7.89E-11	0	0	0.340	0.316
YPX-8	1.05E-09	0	0	0	0.328	0.314
YPX-9	4.67E-10	0	0	0	0.327	0.337
YPX-10	6.39E-10	7.99E-11	0	0	0.320	0.328
YPX-11	5.50E-10	0	0	0	0.334	0.324
YPX-12	4.64E-10	7.74E-11	0	0	0.340	0.329
YPX-13	5.53E-10	7.90E-11	0	0	0.330	0.325
YPX-14	3.24E-10	0	0	0	0.316	0.322
YPX-15	2.44E-10	1.63E-10	0	0	0.324	0.313
YPX-16	4.80E-10	0	0	0	0.328	0.320
YPX-17	8.37E-10	8.37E-11	0	0	0.318	0.301
YPX-18	2.29E-10	1.53E-10	0	0	0.335	0.341
YPX-19	5.45E-10	7.78E-11	0	0	0.334	0.331
YPX-20	1.55E-10	0	0	0	0.328	0.339
YPX-21	2.37E-10	0	0	0	0.337	0.320
YPX-22	4.75E-10	7.91E-11	0	0	0.328	0.326
YPX-23	4.84E-10	0	0	0	0.322	0.319
YPX-24	3.09E-10	7.73E-11	0	0	0.332	0.338

**Table S4. Rates of four types of mutations and growth rate of each sample. Related to Figure 3.** SNV rate and small indel rate shown are numbers of events per nucleotide per cell division, whereas chromosome gain/loss rate and segmental duplication/deletion rate shown are numbers of events per cell division.



Factors being correlated		Factor controlled	Dataset	Pearson's correlation ( <i>P</i> -value)	Spearman's correlation ( <i>P</i> -value)
Growth rate	Mutation rate	Population density	1	-0.61 (0.00057)	-0.71 (<10 <sup>-4</sup> )
Growth rate	Mutation rate	Population density	2	-0.73 (<10 <sup>-4</sup> )	-0.23 (0.16)
Growth rate	Mutation rate	Population density	3	-0.16 (0.30)	-0.37 (0.012)
Population density	Mutation rate	Growth rate	1	0.41 (0.029)	0.48 (0.0096)
Population density	Mutation rate	Growth rate	2	0.51 (0.00098)	0.073 (0.66)
Population density	Mutation rate	Growth rate	3	-0.044 (0.77)	0.080 (0.60)

**Table S5. Partial correlations between population density (or growth rate) and mutation rate upon the control of growth rate (or population density) in three yeast datasets. Related to Figure 3.** Dataset 1: rate of mutation to 5-Fluoro-orotic resistance in strain S288c; Dataset 2: rate of mutation to hygromycin B resistance in strain BY4742; Dataset 3: rate of mutation to hygromycin B resistance in strain Sigma1278b. Data are from [1].

#### SUPPLEMENTAL REFERENCE

1. Krašovec, R., Richards, H., Gifford, D.R., Hatcher, C., Faulkner, K.J., Belavkin, R.V., Channon, A., Aston, E., McBain, A.J., and Knight, C.G. (2017). Spontaneous mutation rate is a plastic trait associated with population density across domains of life. *PLoS Biol* 15, e2002731.