Supporting Information

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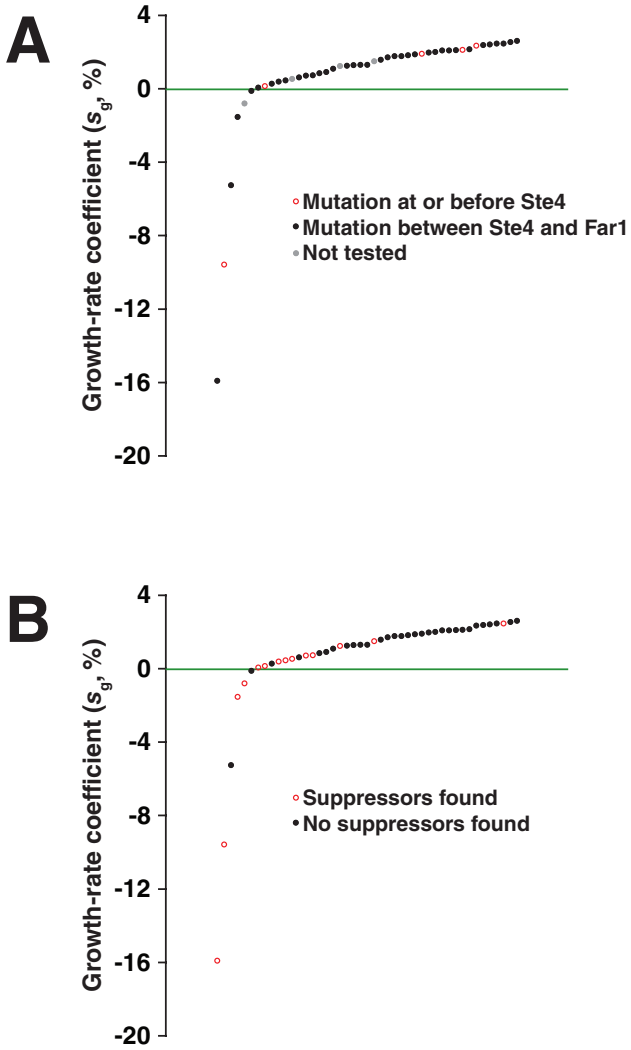


Fig. S1. Phenotypic characterization of the 45 spontaneous αF^R mutants. (*A*) Position of mutations in the mating pathway relative to Ste4 and Far1 was determined by transforming each strain with plasmids containing galactose-inducible *STE4* or *FAR1-22* and assaying for a cell-cycle arrest in the presence of galactose. Two independent transformants were tested for each strain. Strains containing a mutation at or before Ste4 in the mating pathway will arrest after Ste4 overexpression. Such mutations are found throughout the distribution of selection coefficients indicating that a fitness advantage can be gained by losing signaling at multiple points in the mating pathway. All transformed strains arrest after Far1 overexpression; therefore, all of the sterile mutations are within the canonical mating pathway and not downstream of Far1. (*B*) Fourteen of the 45 spontaneous αF^R strains were found to acquire suppressor mutations after as little as 10 generations in YPD as evidenced by the formation of mating projections in response to αF, failure to grow on αF plates, or the restoration of mating. Strains acquiring suppressor mutations are enriched at the lower end of the growth rate distribution ($P = 3.3 \times 10^{-4}$, Wilcoxon). The 4 strains that were not tested in *A* had acquired a suppressor mutation during transformation.

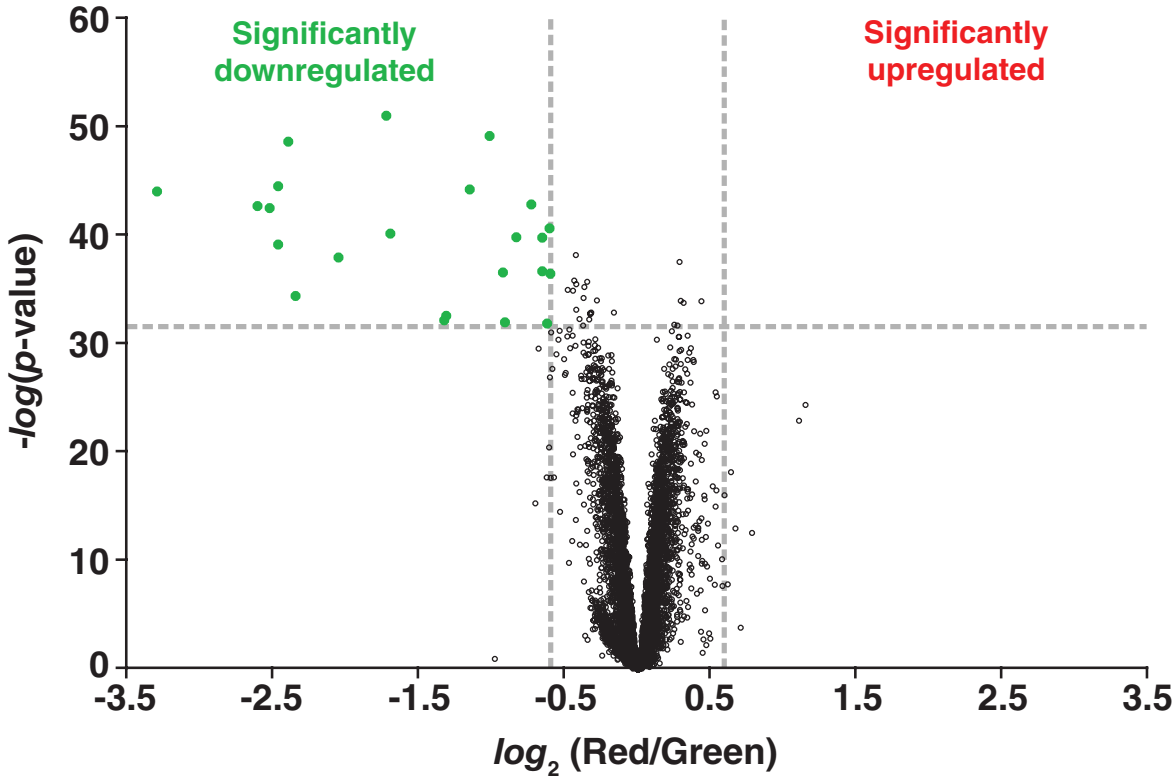


Fig. S2. Identification of significant gene expression changes in the spontaneous αF^R strains with a competitive growth advantage. We extracted the final processed intensities in the Cy3 (green) and Cy5 (red) channels for each probe on the expression microarrays for αF^R-1, -2, -4, -7, -8, -17, and -20, versus the base strain, DBY15084. Because each gene is represented by 7 probes on each of the 7 arrays, we have a total of 49 estimates for the intensity in each channel for every gene on the array. We assayed for significant changes in gene expression by performing a *t* test between the intensities in the 2 channels. Shown is a plot of the $-log(P$ values) versus the log_2(red/green) for each gene. By inspection we defined genes whose expression changes by 1.5-fold or greater with a *P* value $< 10^{-31.5}$ as significant. By these criteria, 23 genes were significantly down-regulated in these strains (green points) with no genes significantly up-regulated.

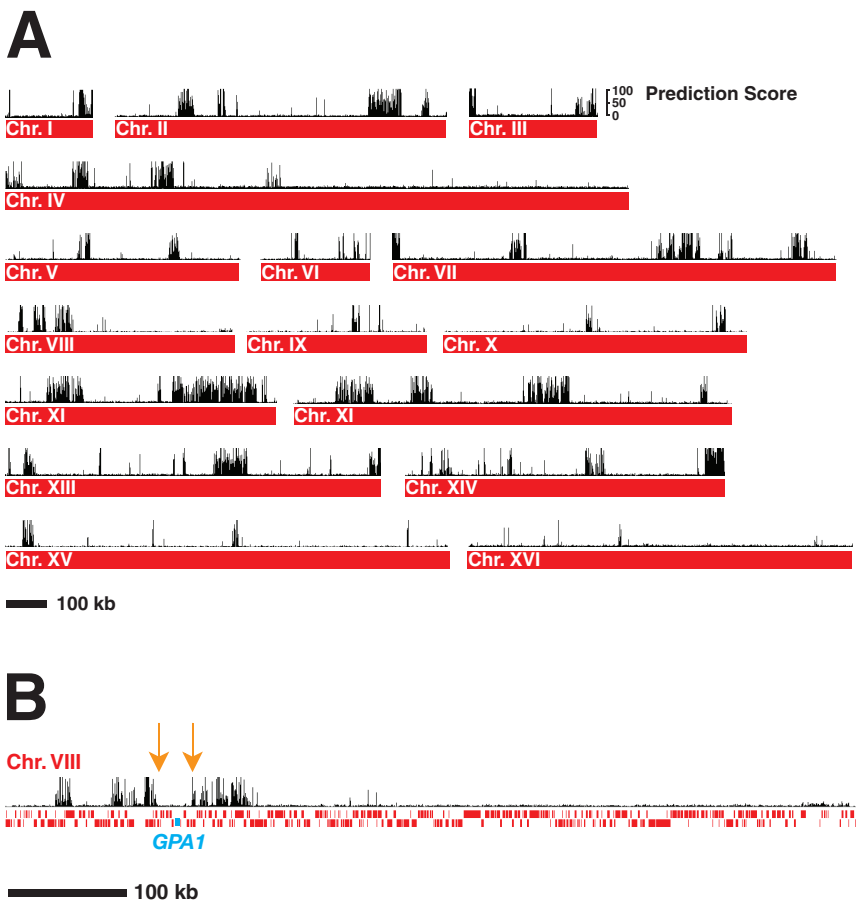
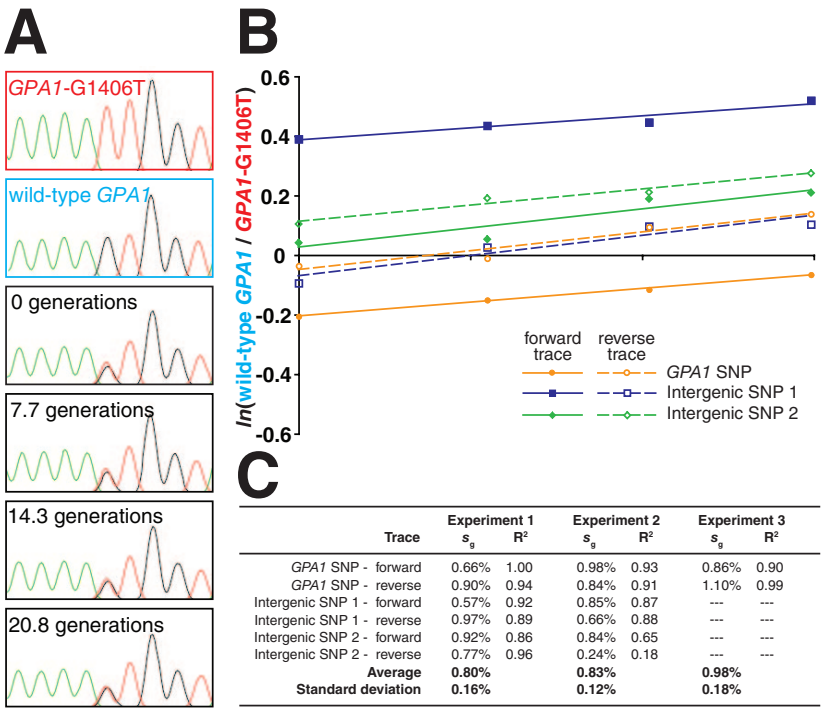


Fig. S3. *GPA1* is located in a region of the W303 genome of predominantly non-S288c descent. (*A*) The W303 genome is a mosaic comprised of ≈85% S288c sequence and contains ≈8,000 single nucleotide polymorphisms (SNPs) relative to the S288c genome (43). SNPs between the W303 base strain, DBY15084, and the sequenced S288c were identified by hybridizing this strain to the Affymetrix yeast tiling array and are represented by peaks above the chromosomes (42). The prediction score is a measure of the likelihood of a genetic difference between the sample and the reference sequence. (*B*) *GPA1* (blue box) is located on the left arm of chromosome VIII in a region of predominantly non-S288c descent; however, there is evidence of recombination breakpoints 8 kb upstream and 14 kb downstream of this gene (orange arrows). The plot of SNPs versus position was generated by using the Integrated Genome Browser (http://www.affymetrix.com). The vertical axis denotes the prediction signal (from 0 to 100 on a linear scale) based on the drop in hybridization intensity of the W303 base strain on the Affymetrix yeast tiling array (42).



Trace	Experiment 1 s_g	Experiment 1 R^2	Experiment 2 s_g	Experiment 2 R^2	Experiment 3 s_g	Experiment 3 R^2
GPA1 SNP - forward	0.66%	1.00	0.98%	0.93	0.86%	0.90
GPA1 SNP - reverse	0.90%	0.94	0.84%	0.91	1.10%	0.99
Intergenic SNP 1 - forward	0.57%	0.92	0.85%	0.87	---	---
Intergenic SNP 1 - reverse	0.97%	0.89	0.66%	0.88	---	---
Intergenic SNP 2 - forward	0.92%	0.86	0.84%	0.65	---	---
Intergenic SNP 2 - reverse	0.77%	0.96	0.24%	0.18	---	---
Average	**0.80%**		**0.83%**		**0.98%**	
Standard deviation	**0.16%**		**0.12%**		**0.18%**	

Fig. S4. Direct competition between strains carrying the wild-type *GPA1* allele and the *GPA1*-G1406T allele validates the conclusion from the FACS-based competitive growth assay that the wild-type *GPA1* allele has a growth-rate advantage of ≈1%. The direct competitions were performed exactly as the relative growth-rate assay except that the ratio of the two strains was determined by quantitative sequencing. (*A*) Shown is a sequencing read across position 1,402–1,410 of *GPA1*. The derived allele of *GPA1* contains a T at position 1,406 (red peak in the middle of the read), whereas the wild-type allele contains a G at this position (black peak in the middle of the read). In a mixed culture both of these peaks are identifiable and their ratio can be observed to change as the culture is passaged over a ≈21 generations. (*B*) Using peak height as a proxy for abundance, we can calculate the growth-rate coefficient (s_g) as the change in the *ln*(wild-type *GPA1* allele/*GPA1*-G1406T allele) versus generations. We performed this analysis on 3 sets of independently transformed strains isogenic except for the *GPA1* allele. In experiments 1 and 2, the strains differ by 2 additional intergenic SNPs that were introduced on the basis of the position of a recombination event during transformation. These SNPs provide additional markers to use for quantitative sequencing. For each time point we amplified the *GPA1* allele and sequenced the product in both directions. This plot shows the change in the allele frequencies versus generations (tick marks on the *x* axis denote 7 generations). Notice that although the slopes for each SNP pair are similar, the *y* intercept varies because of systematic biases arising during sequencing. Therefore, although quantitative sequencing cannot be used to accurately determine the ratio of 2 strains, it can be used effectively to determine the change in the ratio of 2 strains over time. (*C*) The competitive growth advantage (s_g) of the wild-type *GPA1* allele and the correlation coefficient (R^2) for 14 SNP pairs over 3 experiments. The average and standard deviation in experiment 2 does not include the single trace with $R^2 = 0.18$. Excluding this outlier, the overall average competitive growth advantage is indistinguishable from that calculated from the FACS-based assay ($s_g = 0.84\% \pm 0.15\%$ for the direct competition versus $s_g = 0.92\% \pm 0.35\%$ for the FACS-based relative growth-rate assay, $P > 0.10$, *t* test).

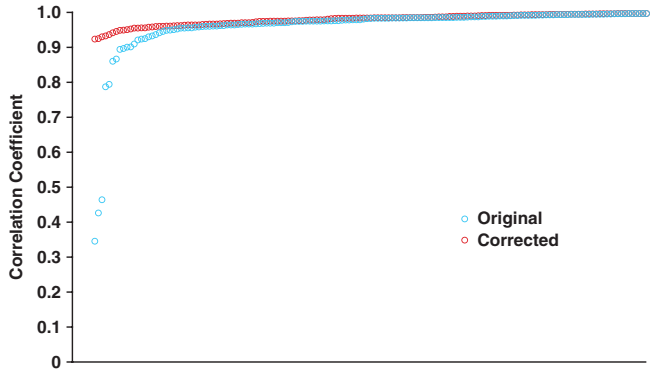


Fig. S5. Correlation coefficient (R^2) values for the 162 growth-rate assays performed in this study. R^2 values are ordered from lowest to greatest. The original R^2 values were calculated by using all 4 data points for each competitive growth assay. For the 13 assays with $R^2 < 0.925$, we found that a single data point was disrupting the trend. The offending data point was removed and s_g and R^2 were recalculated. The most common problem (10 of 13 cases) was that the one strain entered a lag phase after the initial mixing; therefore, the first data point was removed. The values of s_g from the corrected data are reported in this paper.

Table S1. Strains used in this study

Strain name	Genotype	Use
DBY15084	W303 *MAT***a**, *ade2–1*, *CAN1*, *his3–11*, *leu2–3,112*, *trp1–1*, *URA3*, *bar1*Δ::*ADE2*, *hml*α*Δ*::*LEU2*	Base strain
DBY15085	W303 *MAT***a**/*MAT*α, *ade2–1*/*ade2–1*, *his3–11*/*his3–11*, *leu2–3,112* /*leu2–3,112*, *trp1–1*/*trp1–1*, *CWP2*/*CWP2*-YFP(yEVenus)::HphMX	FACS reference
DBY15086	W303 *MAT***a**, *ade2–1*, *CAN1*, *his3–11*, *leu2–3,112*, *trp1–1*, *URA3*, *bar1*Δ::*ADE2*, *hml*α*Δ*::*LEU2*, *ste2*Δ::KanMX	Growth-rate assay
DBY15087	W303 *MAT***a**, *ade2–1*, *CAN1*, *his3–11*, *leu2–3,112*, *trp1–1*, *URA3*, *bar1*Δ::*ADE2*, *hml*α*Δ*::*LEU2*, *ste4*Δ::KanMX	Growth-rate assay
DBY15088	W303 *MAT***a**, *ade2–1*, *CAN1*, *his3–11*, *leu2–3,112*, *trp1–1*, *URA3*, *bar1*Δ::*ADE2*, *hml*α*Δ*::*LEU2*, *ste7*Δ::KanMX	Growth-rate assay
DBY15089	W303 *MAT***a**, *ade2–1*, *CAN1*, *his3–11*, *leu2–3,112*, *trp1–1*, *URA3*, *bar1*Δ::*ADE2*, *hml*α*Δ*::*LEU2*, *ste12*Δ::KanMX	Growth-rate assay
DBY15090	W303 *MAT***a**, *ade2–1*, *CAN1*, *his3–11*, *leu2–3,112*, *trp1–1*, *URA3*, *bar1*Δ::*ADE2*, *hml*α*Δ*::*LEU2*, *far1*Δ::KanMX	Growth-rate assay
DBY15091	W303 *MAT***a**, *ade2–1*, *CAN1*, *his3–11*, *leu2–3,112*, *trp1–1*, *URA3*, *bar1*Δ::*ADE2*, *hml*α*Δ*::*LEU2*, *can1*Δ::KanMX	Growth-rate assay
DBY15092	W303 *MAT***a**, *ade2–1*, *CAN1*, *his3–11*, *leu2–3,112*, *trp1–1*, *URA3*, *bar1*Δ::*ADE2*, *hml*α*Δ*::*LEU2*, *GPA1*-G1406T::NatMX	Growth-rate assay
DBY15093	W303 *MAT***a**, *ade2–1*, *CAN1*, *his3–11*, *leu2–3,112*, *trp1–1*, *URA3*, *bar1*Δ::*ADE2*, *hml*α*Δ*::*LEU2*, *GPA1*-G1406T::NatMX	Growth-rate assay
DBY15094	W303 *MAT***a**, *ade2–1*, *CAN1*, *his3–11*, *leu2–3,112*, *trp1–1*, *URA3*, *bar1*Δ::*ADE2*, *hml*α*Δ*::*LEU2*, *GPA1*-G1406T::NatMX	Growth-rate assay
DBY15095	W303 *MAT***a**, *ade2–1*, *CAN1*, *his3–11*, *leu2–3,112*, *trp1–1*, *URA3*, *bar1*Δ::*ADE2*, *hml*α*Δ*::*LEU2*, *GPA1*^{WT}::NatMX	Growth-rate assay
DBY15096	W303 *MAT***a**, *ade2–1*, *CAN1*, *his3–11*, *leu2–3,112*, *trp1–1*, *URA3*, *bar1*Δ::*ADE2*, *hml*α*Δ*::*LEU2*, *GPA1*^{WT}::NatMX	Growth-rate assay
DBY15097	W303 *MAT***a**, *ade2–1*, *CAN1*, *his3–11*, *leu2–3,112*, *trp1–1*, *URA3*, *bar1*Δ::*ADE2*, *hml*α*Δ*::*LEU2*, *GPA1*^{WT}::NatMX	Growth-rate assay
DBY15098	W303 *MAT*α, *ade2–1*, *can1–100*, *HIS3*, *leu2–3,112*, *TRP1*,*URA3*	Strain construction
DBY15099	W303 *MAT***a**, *ade2–1*, *CAN1*, *his3–11*, *leu2–3,112*, *trp1–1*, *URA3*, *hml*α*Δ*::*LEU2*, *GPA1*-G1406T::NatMX	Mating assay
DBY15100	W303 *MAT***a**, *ade2–1*, *CAN1*, *his3–11*, *leu2–3,112*, *trp1–1*, *URA3*, *hml*α*Δ*::*LEU2*, *GPA1*-G1406T::NatMX	Mating assay
DBY15101	W303 *MAT***a**, *ade2–1*, *CAN1*, *his3–11*, *leu2–3,112*, *trp1–1*, *URA3*, *hml*α*Δ*::*LEU2*, *GPA1*^{WT}::NatMX	Mating assay
DBY15102	W303 *MAT***a**, *ade2–1*, *CAN1*, *his3–11*, *leu2–3,112*, *trp1–1*, *URA3*, *hml*α*Δ*::*LEU2*, *GPA1*^{WT}::NatMX	Mating assay
DBY15103	W303 *MAT*α, *ade2–1*, *can1–100*, *HIS3*, *leu2–3,112*, *TRP1*,*URA3*, *bar1*Δ::*ADE2*	Mating assay

Table S2. Primers used in this study

Primer name	Sequence (5′ → 3′)	Use
STE2extF1	ATTTAAGCAGGCCAACGTCC	Strain construction
STE2extR1	CTGAGAGTTCTAGATCATGG	Strain construction
STE4extF1	CTTCACATGACTTGAATCCC	Strain construction/sequencing
STE4extR1	TAGATGATTCTAGCAAGTTGG	Strain construction/sequencing
STE7extF1	AGTTCTAAGATTGTGTTGTCC	Strain construction
STE7extR1	GGGTTATTAATCGCCTTCGG	Strain construction
STE12extF1	ACATAGTACCACTACGTTCC	Strain construction
STE12extR1	CGATCATGTAGTTTTGGAGG	Strain construction
FAR1extF1	CAAATGCGAAGGTACCTTGG	Strain construction
FAR1extR1	GCCAATAGGTTCTTTCTTAGG	Strain construction
CAN1extF1	TAAACCGAATCAGGGAATCC	Strain construction
CAN1extR1	CGGGAGCAAGATTGTTGTGG	Strain construction
KanMX/NatMXintR	CCTTAATTAACCCGGGGATC	Strain verification/sequencing
STE2extF2	GTCTCGTGCATTAAGACAGG	Strain verification
STE4extF2	GTTTTCAACTGACACTTGCC	Strain verification
STE7extF2	GTGGTCTAAAAAGAATGTGG	Strain verification
STE12extF2	ACAACTCTTCGCGGTCAGG	Strain verification
FAR1extF2	GCCATTTCACCGAAAATACC	Strain verification
CAN1extF2	AATCTAGGGTTTCTGTGTGG	Strain verification
SNP1_APC1_F	GTTTCAAAATCTTGGGCTTGT	Sequencing
SNP1_APC1_R	TGGGCATATTCTGTCTTGGTG	Sequencing
SNP2_EDS1_F	CGGTTCCGCTCAATTTTATC	Sequencing
SNP2_EDS1_R	ACACTGCCCCTTTTTTTTGC	Sequencing
SNP3_SUM1_F	GCCTGCCTCTTTTTCTTTTG	Sequencing
SNP3_SUM1_R	CACACAACCAATCAGTTTTGG	Sequencing
SNP4_STE7_F	GGGCTAAAAGTAAGAATTTTC	Sequencing
SNP4_STE7_R	ACGGGGATTGTGATAGAGAA	Sequencing
SNP5_MEF2_F	CAACAAAAATCTTTGCATTGC	Sequencing
SNP5_MEF2_R	CGTTTGGCCAGTCTCATCATT	Sequencing
SNP6_CPD1_F	TGTAGGCGTGATCAAGTGCTT	Sequencing
SNP6_CPD1_R	CACTCGAGCACAATTTTCTTG	Sequencing
SNP7_STE11_F	GGCAAATACTGATTGGGGTT	Sequencing
SNP7_STE11_R	CCAGGGGTATGAGAATCAAAT	Sequencing
SNP8_STE5_F	TACACCTTCTACGGTTTCCA	Sequencing
SNP8_STE5_R	TCGTTTGTCTTTCAACAGG	Sequencing
SNP9_IKS1_F	GTGGTTCAAAGGGCAATTCA	Sequencing
SNP9_IKS1_R	TACAACAGTATTGCAGTGGCG	Sequencing
STE4intF1	CATATATTGACAGCAAGTGG	Sequencing
STE4intR1	AGAAATATAGCAAGTATGTCC	Sequencing
GPA1_intF1_HindIII	AGTTTTATTAAGCTTAGCAATGAGTGAATACGACC	Plasmid construction
GPA1_extR1_SacII	TACCAGGGACCGCGGTTCGAGATAATACCCTGTCC	Plasmid construction
GPA1intF2	AATTTAACATCGGCTCGTCC	Sequencing
GPA1extR2	ATATATCCCGAGTATTTACC	Sequencing