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Note

Precise Gene-Dose Alleles for Chemical Genetics

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ABSTRACT

Modulating gene dose is an effective way to alter protein levels and modify phenotypes to understand gene function. In addition, combining gene-dose alleles with chemical perturbation can provide insight into drug–gene interactions. Here, we present a strategy that combines diverse loss-of-function alleles to systematically modulate gene dose in *Saccharomyces cerevisiae*. The generated gene dosage allele set expands the genetic toolkit for uncovering novel phenotypes.

ALTERING gene dose is a well-established means to perturb and thereby infer gene function. In yeast, different alleles that manifest different gene doses such as homozygous deletions (Lee *et al.* 2005), heterozygous deletions (Giaever *et al.* 2002), overexpression alleles (Sopko *et al.* 2006), and other conditional alleles (Mnaimneh *et al.* 2004; Ben-Aroya *et al.* 2008) have been used toward this end. Altering gene dose alone is not, however, always sufficient to induce a detectable phenotypic change. For example, ~64% of homozygous yeast deletion strains and 97% of heterozygote yeast deletion strains manifest wild-type fitness in optimal growth conditions (Winzeler *et al.* 1999; Giaever *et al.* 2002; Deutschbauer *et al.* 2005), suggesting that additional perturbations may be required to uncover a phenotype. This limitation can be circumvented by combining gene-dose alleles with chemical perturbation. A recent study reported that nearly all gene deletions can exhibit a condition-dependent growth phenotype (Hillenmeyer *et al.* 2008), supporting the hypothesis that chemical perturbation combined with genetic lesions can be a generally useful approach.

To combine gene dose and compound dose to identify gene function one requires a set of well-annotated compounds with known mechanisms of action. Unfortunately, the protein targets of many compounds are still, in large part, unknown and compound selection remains a challenge. Although several yeast genomics-based strategies have been developed to identify drug targets (Sturgeon *et al.* 2006), each has certain limitations. For example, **h**aplo**i**nsufficiency **p**rofiling (HIP) represents an assay with the potential to reveal drug targets (Giaever *et al.* 1999, 2002, 2004; Baetz *et al.* 2004; Lum *et al.* 2004; Doostzadeh *et al.* 2007) but it can fail to identify drug targets if the reduction in gene dose is insufficient. A new pooled assay uses a collection of Decreased Abundance by mRNA Perturbation (DAmP) loss-of-function alleles to identify drug targets (Yan *et al.* 2008). "DAmPing" involves disrupting the 3′-untranslated region immediately downstream of a gene's stop codon, resulting in the destabilization of the gene's mRNA and decreasing the amount of protein. In practice, protein abundance is decreased to ~5–50% of wild-type levels (Schuldiner *et al.* 2005). The DAmP assay can be more sensitive than the HIP assay and has identified several drug targets that were missed by the HIP assay. The DAmP assay is not without limitations. First, only 87.1% of yeast essential genes are represented as DAmP alleles, and the remaining 13% of essential genes are probably inviable as DAmP alleles. Second, of the 958 viable DAmP alleles, 17% manifest fitness defects in rich media, potentially complicating competitive growth assays. Interestingly, when we analyzed DAmP-inviable and DAmP-sick strains and compared them to haploinsufficient heterozygous strains, we found that 47.6% (59/124) of slow-growing heterozygous strains were also slow growing as DAmP strains, suggesting that

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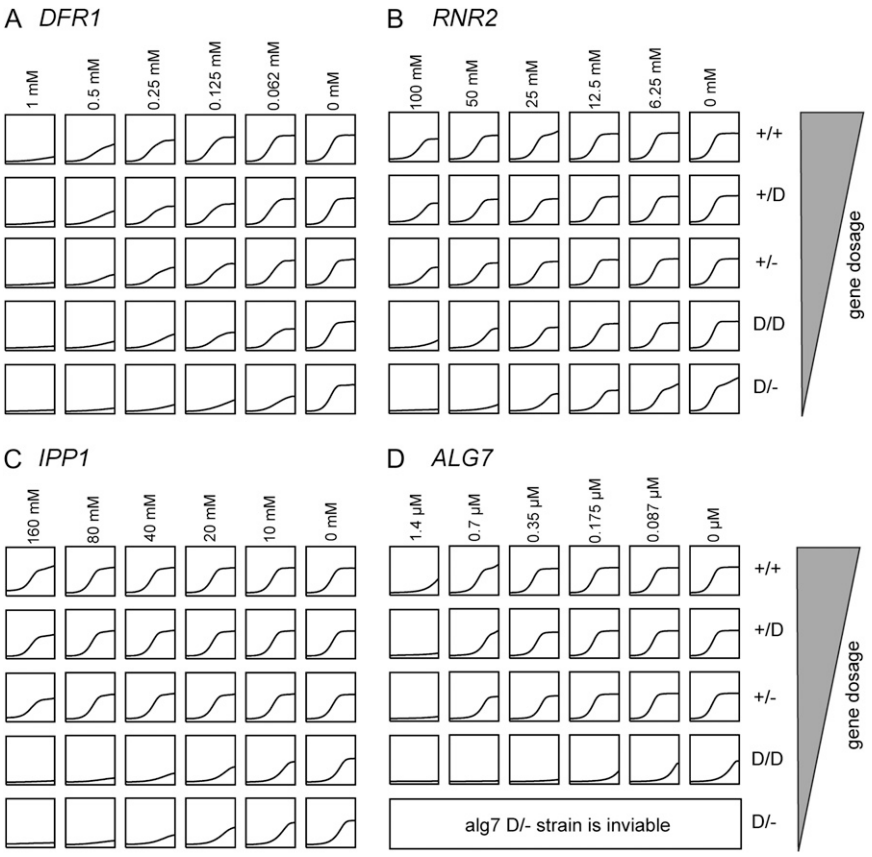

FIGURE 1.—Drug sensitivity of gene-dose strains. Each column represents a different drug concentration with concentration indicated at the top. Each row represents a different gene-dose strain with the strain genotype indicated at the right. (A) Growth curve of different *DFR1* gene-dose strains. Each strain was grown in YPD medium with or without methotrexate. (B) Growth curve of different *RNR2* gene-dose strains. Each strain was grown in YPD medium with or without hydroxyurea. (C) Growth curve of different *IPP1* gene-dose strains. Each strain was grown in YPD medium with or without NaF. (D) Growth curve of different *ALG7* gene-dose strains. Each strain was grown in YPD medium with or without tunicamycin. Strains and genotypes are listed in Table S1. The diploid parental wild-type strain is BY4743. All DAmP and heterozygous strains were created as described (GIAEVER *et al.* 2002; SCHULDINER *et al.* 2005). Primers used to create gene-dose strains are listed in Table S2. Each primer contains 40 bases of homology to its target gene and 18 bases of homology to the KanMX4 or NatMX4 cassette. To create double DAmP strains, two haploid DAmP strains were created, one with the 3′-UTR disrupted using a KanMX4 cassette in strain BY4741 and the other with a NatMX4 cassette in strain BY4742. Haploid DAmP strains were mated and diploids selected on YPD medium containing 250 mg/liter of G418 (AgriBio, no. 3000) and 100 mg/liter of nourseothricin (Werner Bioagents). The hemizygous DAmP strains (D/− allele) were constructed by inserting the NatMX4 cassette immediately after the stop codon of the gene of interest in a heterozygous strain and were selected on YPD medium containing 250 mg/liter of G418 and 100 mg/liter of nourseothricin.

haploinsufficiency and "DAmP insufficiency" share mechanistic similarities.

We reasoned that, by combining heterozygous alleles with DAmP alleles, we could produce cells or "allele sets" that span a wide range of gene doses and thereby improve the sensitivity of genome-based assays. Toward this end, we developed a strategy to systematically adjust gene dose in yeast. As a proof-of-principle, we

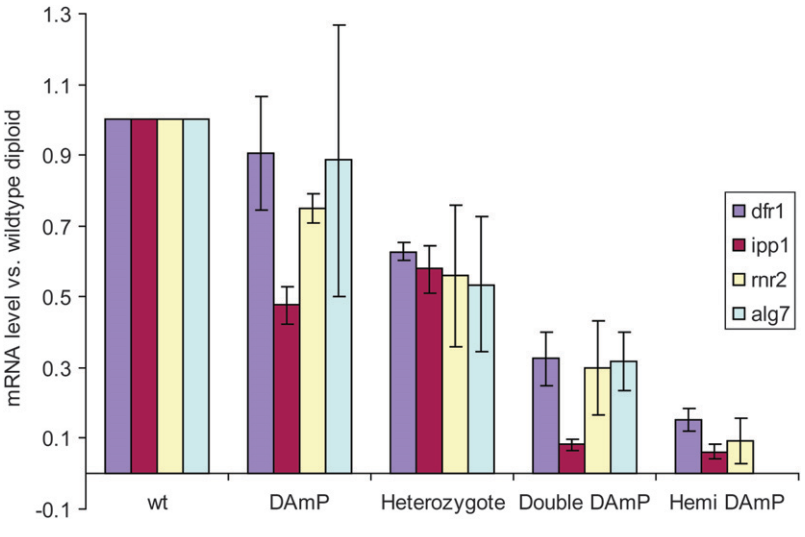

FIGURE 2.—mRNA expression level in gene-dose strains. The mRNA expression level of each gene was determined by quantitative RT–PCR. Histograms represent the expression level of each gene in the gene-dosage strain relative to that of the wild-type strain. Each sample was assayed in triplicate. The bar in each histogram represents the standard deviation. The *ALG7* D/− strain was not viable; therefore only four strains are shown in the *ALG7* gene-dose allele set. To measure RNA level, exponentially growing yeast cells were collected and RNA was extracted using the RNeasy mini kit (QIAGEN, Valencia, CA; no. 74104). Ten micrograms of total RNA from each strain were treated with turbo DNase (Ambion, no. AM1907) to remove residual genomic DNA. cDNA was reverse transcribed according to manufacturer's instructions (Invitrogen, Carlsbad, CA). Quantitative real-time PCR was performed using SYBR green chemistry (Applied Biosystems, Foster City, CA; no. 4367660) for 40 cycles at an annealing temperature of 60°. The primers used are listed in Table S2. Each sample was assayed in triplicate. mRNA expression levels were normalized relative to the endogenous gene *ACT1*. PCR efficiency was corrected using the LinRegPCR protocol (RAMAKERS *et al.* 2003). The expression level of each strain relative to the wild-type control was then calculated according to the $2 - \Delta\Delta Ct$ method (LIVAK and SCHMITTGEN 2001).

TABLE 1

Compounds and their putative protein targets

Drugs/compounds	Target
Methotrexate	DFR1
NaF	IPP1
Hydroxyurea	RNR2
Tunicamycin	ALG7

created four alleles for several well-characterized essential genes in which gene dose was modulated from (on average) 5 to 100% that of wild type. These strains showed gene-dose-dependent, drug-sensitive phenotypes and experiments with these allele sets using reference compounds show that modulating gene dose, when combined with compound titrations, provides a highly sensitive means to dissect drug–gene interactions.

These gene-dose allele sets comprise five diploid strains (wild type and four mutants), each with a different gene dose: (1) a wild-type diploid strain (WT), (2) a diploid DAmP strain (D/+) that contains one wild-type allele and one DAmP allele, (3) a heterozygous diploid strain (−/+) that contains only one wild-type allele, (4) a diploid double DAmP strain (D/D) that contains two DAmP alleles, and (5) a hemizygous DAmP strain (−/D) that only contains a single DAmP allele. The gene doses of these five strains are predicted to be 1, 0.525–0.75, 0.5, 0.05–0.5, and 0.025–0.25, respectively. These gene doses are based on the following assumptions: (1) heterozygous alleles will be, on average, 50% of the WT gene dose, and a typical DAmP allele will produce 5–50% of wild-type protein levels (Schuldiner *et al.* 2005). The actual gene dose for any one strain will, of course, deviate from these ideal expectations. We created four gene-dose alleles for the following genes: *DFR1*, *ALG7*, *RNR2*, and *IPP1*. Of 20 possible strains (Table S1), we were able to create 19 and failed to create the *alg7* −/D strain, most likely due to the fact that this allele produces too little protein for viability. Indeed the *alg7* D/D strain is quite sick, suggesting that this strain produces the minimum amount of gene product required to sustain viability (Figure 1D). All strains were confirmed by PCR with primers flanking the stop codon of each gene and primers flanking the antibiotic gene junction region (Figure S1).

To verify the level of gene dose in each strain, we measured the mRNA levels for each strain using quantitative reverse transcriptase PCR (qRT–PCR). As shown in Figure 2, the wild-type strain had a full complement of RNA per locus (by definition WT = 100% of WT levels) and each allele in the gene-dose allele set had a decreasing amount of mRNA. Levels follow this trend: the WT strain > D/+ strain > the heterozygous −/+ strain > the D/D strain > −/D strain. To test if these gene-dose sets can be useful for assessing drug sensitivity, we treated each allele set strain with a compound known to inhibit the protein product encoded by the altered locus (Table 1). In each case, strains were more sensitive to drug as a function of gene dose (Figure 1).

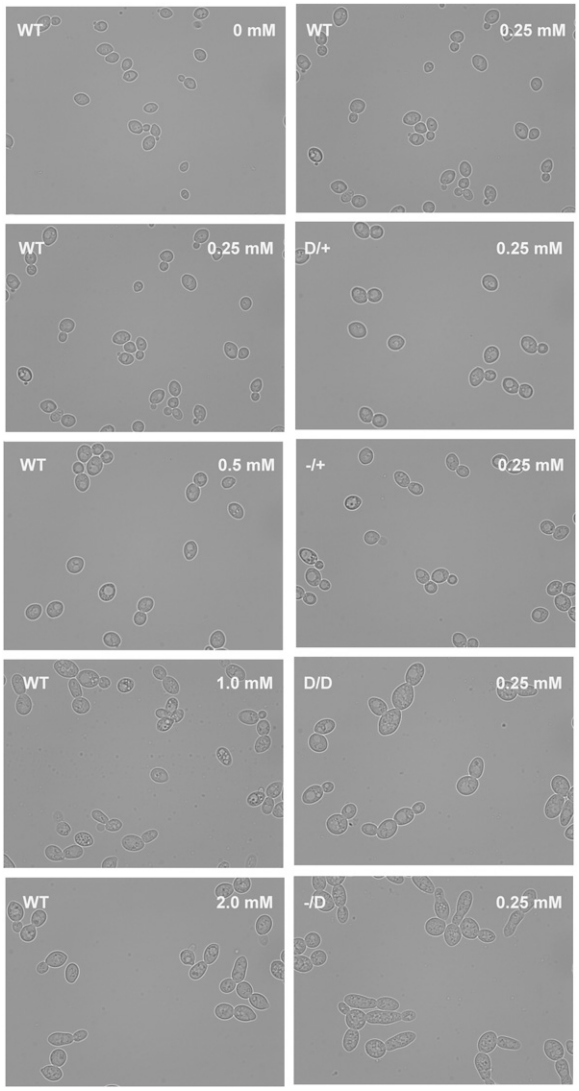

Figure 3.—Morphological comparison of gene-dosage response to drug-dosage response: morphology of the wild-type strain treated with different concentrations of methotrexate in YPD medium (left column) and morphology of different *DFR1* gene-dose strains grown in 250 μM of methotrexate (right column). Cells were grown in YPD with or without methotrexate at 30° for 2 days. Cells were then harvested and observed live with a Zeiss Axiovert 200 microscope using a 63× objective. Images were collected using bright field illumination, using an Axiocam HR.

A significant limitation of the current chemical genetics approach is that compounds are often expensive and can be limited in availability. In addition, small molecules can interact with multiple protein targets, which can confound determination of the protein function. To ask if our gene-dose alleles could address some of these limitations, we combined different *DFR1* gene-dose alleles with 250 μM methotrexate, a drug that targets DFR1.

Methotrexate treatment resulted in a phenotype characterized by enlarged and elongated cells, apparently a result of a defect in cytokinesis. Interestingly, as the gene dose of *DFR1* was lowered, this phenomenon became more pronounced (Figure 3). Although similar morphological changes were observed when wild-type cells were treated with methotrexate, they required very high doses (>500 μM) of compound. In addition, the elongated cells in the drug-treated *DFR1* −/D strain are more pronounced than those seen in methotrexate-treated wild-type cells, even for WT cells treated by 2 mM of methotrexate. Higher methotrexate doses were lethal for WT yeast cells due to nonspecific toxicity. These results suggest that (1) lowering gene dose either through genetic or chemical perturbation can cause a similar phenotype, (2) gene-dose alleles permit a greater dynamic range within which one can observe a morphological defect, and (3) gene-dose alleles can complement gene–drug interaction studies by amplifying drug-induced phenotypes and can simplify the determination of the appropriate gene dose and drug concentration to amplify the phenotype of interest.

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LITERATURE CITED

Baetz, K., L. McHardy, K. Gable, T. Tarling, D. Reberioux *et al.*, 2004 Yeast genome-wide drug-induced haploinsufficiency screen to determine drug mode of action. Proc. Natl. Acad. Sci. USA **101:** 4525–4530.

Ben-Aroya, S., C. Coombes, T. Kwok, K. A. O'Donnell, J. D. Boeke *et al.*, 2008 Toward a comprehensive temperature-sensitive mutant repository of the essential genes of Saccharomyces cerevisiae. Mol. Cell **30:** 248–258.

Deutschbauer, A. M., D. F. Jaramillo, M. Proctor, J. Kumm, M. E. Hillenmeyer *et al.*, 2005 Mechanisms of haploinsufficiency revealed by genome-wide profiling in yeast. Genetics **169:** 1915–1925.

Doostzadeh, J., R. W. Davis, G. N. Giaever, C. Nislow and J. W. Langston, 2007 Chemical genomic profiling for identifying intracellular targets of toxicants producing Parkinson's disease. Toxicol. Sci. **95:** 182–187.

Giaever, G., D. D. Shoemaker, T. W. Jones, H. Liang, E. A. Winzeler *et al.*, 1999 Genomic profiling of drug sensitivities via induced haploinsufficiency. Nat. Genet. **21:** 278–283.

Giaever, G., A. M. Chu, L. Ni, C. Connelly, L. Riles *et al.*, 2002 Functional profiling of the Saccharomyces cerevisiae genome. Nature **418:** 387–391.

Giaever, G., P. Flaherty, J. Kumm, M. Proctor, C. Nislow *et al.*, 2004 Chemogenomic profiling: identifying the functional interactions of small molecules in yeast. Proc. Natl. Acad. Sci. USA **101:** 793–798.

Hillenmeyer, M. E., E. Fung, J. Wildenhain, S. E. Pierce, S. Hoon *et al.*, 2008 The chemical genomic portrait of yeast: uncovering a phenotype for all genes. Science **320:** 362–365.

Lee, W., R. P. St. Onge, M. Proctor, P. Flaherty, M. I. Jordan *et al.*, 2005 Genome-wide requirements for resistance to functionally distinct DNA-damaging agents. PLoS Genet. **1:** e24.

Livak, K. J., and T. D. Schmittgen, 2001 Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta Delta C(T)). Methods **25:** 402–408.

Lum, P. Y., C. D. Armour, S. B. Stepaniants, G. Cavet, M. K. Wolf *et al.*, 2004 Discovering modes of action for therapeutic compounds using a genome-wide screen of yeast heterozygotes. Cell **116:** 121–137.

Mnaimneh, S., A. P. Davierwala, J. Haynes, J. Moffat, W. T. Peng *et al.*, 2004 Exploration of essential gene functions via titratable promoter alleles. Cell **118:** 31–44.

Ramakers, C., J. M. Ruijter, R. H. Deprez and A. F. Moorman, 2003 Assumption-free analysis of quantitative real-time polymerase chain reaction (PCR) data. Neurosci. Lett. **339:** 62–66.

Schuldiner, M., S. R. Collins, N. J. Thompson, V. Denic, A. Bhamidipati *et al.*, 2005 Exploration of the function and organization of the yeast early secretory pathway through an epistatic miniarray profile. Cell **123:** 507–519.

Sopko, R., D. Huang, N. Preston, G. Chua, B. Papp *et al.*, 2006 Mapping pathways and phenotypes by systematic gene overexpression. Mol. Cell **21:** 319–330.

Sturgeon, C. M., D. Kemmer, H. J. Anderson and M. Roberge, 2006 Yeast as a tool to uncover the cellular targets of drugs. Biotechnol. J. **1:** 289–298.

Winzeler, E. A., D. D. Shoemaker, A. Astromoff, H. Liang, K. Anderson *et al.*, 1999 Functional characterization of the S. cerevisiae genome by gene deletion and parallel analysis. Science **285:** 901–906.

Yan, Z., M. Costanzo, L. E. Heisler, J. Paw, F. Kaper *et al.*, 2008 Yeast barcoders: a chemogenomic application of a universal donor-strain collection carrying bar-code identifiers. Nat. Methods **5:** 719–725.

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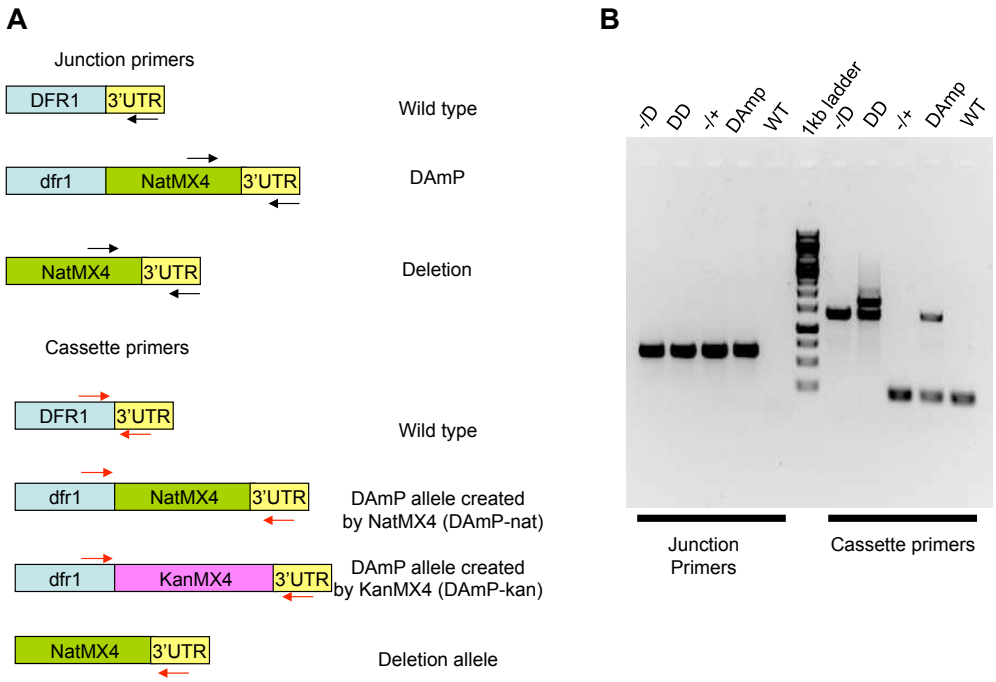

FIGURE S1.—Confirmation of gene-dose strains by PCR. The *DFR1* gene-dose alleles were used to illustrate the confirmation of gene-dose alleles. (A) Schematic diagram of primers used to confirm gene dosage alleles. Two pairs of primers were used to confirm gene dose strains. One pair spans the right junction of the mutation module (junction primer) represented by black short arrows in the figure. A second pair of primers flanks the stop codon of *DFR1* (cassette primer) represented by red short arrows. Using this pair of primers, the deletion modules cannot produce a product because the homologous region of one of the primers was deleted. The other modules can produce a PCR product but the size of PCR product varies depending on modules. The size of PCR products for the wild type allele, DAmP –Kan and DAmP-Nat allele are 207, 1676 and 1326 base pair respectively. (B) Agarose gel analysis of confirmation PCR of *DFR1* gene-dose strains. The strain names were listed in the top of each lane. When using the junction primer pair (left side), the D/+, D/D, -/D and -/+ strain produced a 650 base pair PCR product whereas the wild type strain will not generate a PCR product. When using the cassette primer (right side), the -/D produced a 1326 bp band. The wild type and -/+ strain produced a 207 bp band. The D/+ strain produced two bands, 1326 bp and 207 bp. The D/D strain produced two bands, 1326 bp and 1676 bp. To verify the genotype of each strain, genomic DNA was prepared using the YeaStar genomic DNA kit (Zymo research, cat# D2002) and used as template in PCR. Two pairs of primers were used to confirm each strain (Table S2).

TABLE S1

Strains used in this study

Name	Genotype	Source
BY4741	*MAT***a** *his3Δ1 leu2Δ0 ura3Δ0 met15Δ0*	Boone lab
BY4742	*MATα his3Δ1 leu2Δ0 ura3Δ0 lys2Δ0*	Boone lab
BY4743	his3Δ1/his3Δ1, leu2Δ0/leu2Δ0, ura3Δ0/ura3Δ0,met15Δ0/+, lys2Δ0/+	This study
dfr1 D/+	*his3Δ1/his3Δ1, leu2Δ0/leu2Δ0, ura3Δ0/ura3Δ0,met15Δ0/+, lys2Δ0/+, dfr1-DAmP::natMX4/+*	This study
dfr1 -/+	*his3Δ1/his3Δ1, leu2Δ0/leu2Δ0, ura3Δ0/ura3Δ0,met15Δ0/+, lys2Δ0/+, dfr1Δ0::natMX4/+*	This study
dfr1 D/D	*his3Δ1/his3Δ1, leu2Δ0/leu2Δ0, ura3Δ0/ura3Δ0,met15Δ0/+, lys2Δ0/+, dfr1-DAmP::natMX4/dfr1-DAmP::kanMX4*	This study
dfr1 -/D	*his3Δ1/his3Δ1, leu2Δ0/leu2Δ0, ura3Δ0/ura3Δ0,met15Δ0/+, lys2Δ0/+, dfr1-DAmP::natMX4/-*	This study
rnr2 D/+	*his3Δ1/his3Δ1, leu2Δ0/leu2Δ0, ura3Δ0/ura3Δ0,met15Δ0/+, lys2Δ0/+, rnr2-DAmP::natMX4/+*	This study
rnr2 -/+	*his3Δ1/his3Δ1, leu2Δ0/leu2Δ0, ura3Δ0/ura3Δ0,met15Δ0/+, lys2Δ0/+, rnr2Δ0::natMX4/+*	This study
rnr2 D/D	*his3Δ1/his3Δ1, leu2Δ0/leu2Δ0, ura3Δ0/ura3Δ0,met15Δ0/+, lys2Δ0/+, rnr2-DAmP::natMX4/rnr2-DAmP::kanMX4*	This study
rnr2 -/+	*his3Δ1/his3Δ1, leu2Δ0/leu2Δ0, ura3Δ0/ura3Δ0,met15Δ0/+, lys2Δ0/+, rnr2-DAmP::natMX4/-*	This study
ipp1 D/+	*his3Δ1/his3Δ1, leu2Δ0/leu2Δ0, ura3Δ0/ura3Δ0,met15Δ0/+, lys2Δ0/+, ipp1-DAmP::natMX4/+*	This study
ipp1 -/+	*his3Δ1/his3Δ1, leu2Δ0/leu2Δ0, ura3Δ0/ura3Δ0,met15Δ0/+, lys2Δ0/+,ipp1Δ0::natMX4/+*	This study
ipp1 D/D	*his3Δ1/his3Δ1, leu2Δ0/leu2Δ0, ura3Δ0/ura3Δ0,met15Δ0/+, lys2Δ0/+, ipp1-DAmP::natMX4/ipp1-DAmP::kanMX4*	This study
ipp1 -/D	*his3Δ1/his3Δ1, leu2Δ0/leu2Δ0, ura3Δ0/ura3Δ0,met15Δ0/+, lys2Δ0/+, ipp1-DAmP::kanMX4/-*	This study
alg7 D/+	*his3Δ1/his3Δ1, leu2Δ0/leu2Δ0, ura3Δ0/ura3Δ0,met15Δ0/+, lys2Δ0/+, alg7-DAmP::natMX4/+*	This study
alg7 -/+	*his3Δ1/his3Δ1, leu2Δ0/leu2Δ0, ura3Δ0/ura3Δ0,met15Δ0/+, lys2Δ0/+, alg7Δ0::natMX4/+*	This study
alg7 -/D	*his3Δ1/his3Δ1, leu2Δ0/leu2Δ0, ura3Δ0/ura3Δ0,met15Δ0/+, lys2Δ0/+, alg7-DAmP::natMX4/alg7-DAmP::kanMX4*	This study

TABLE S2

Primers used in this study

Gene name	Sequence (5'-3')	Comments
DFR1	AGGTTATTGCTTCGAATTCACTCTATACAATCGT AAATGACGTACGCTGCAGGTCGAC	Forward primer used to create *dfr1-DAmP::kanMX4* allele
DFR1	CTATTTAACATATTAAAAAAAATATACGGGCGGA GAGGTTATCGATGAATTCGAGCTCG	Reverse primer used to create *dfr1-DAmP::kanMX4* or *dfr1Δ0::kanMX4* allele
DFR1	TGATAATAAGAGAAATTGAAGAGCGCAACGAACT ACGAGCCGTACGCTGCAGGTCGAC	Forward primer used to create *dfr1Δ0::kanMX4* allele
DFR1	AGGTTATTGCTTCGAATTCACTCTATACAATCGT AAATGAACATGGAGGCCCAGAATACCC	Forward primer used to create *dfr1-DAmP::natMX4* allele
DFR1	CTATTTAACATATTAAAAAAAATATACGGGCGGA GAGGTTCAGTATAGCGACCAGCATTCAC	Reverse primer used to create *dfr1-DAmP::natMX4* or *dfr1Δ0::natMX4* allele
DFR1	TGATAATAAGAGAAATTGAAGAGCGCAACGAACT ACGAGCACATGGAGGCCCAGAATACCC	Forward primer used to create *dfr1Δ0::natMX4* allele
IPP1	GTCTATTGACAAGTGGTTCTTCATCTCCGGTTCT GTTTAACGTACGCTGCAGGTCGAC	Forward primer used to create *ipp1-DAmP::kanMX4* allele
IPP1	CGCATACGTGGTAGGTGTCTCATTTTCAATTCAA AATATTATCGATGAATTCGAGCTCG	Reverse primer used to create *ipp1-DAmP::kanMX4* or *ipp1Δ0::kanMX4* allele
IPP1	TAGGTCTATAGAACAGGATATCCCGCCGCGCAAT TTACTACGTACGCTGCAGGTCGAC	Forward primer used to create *ipp1Δ0::kanMX4* allele
IPP1	GTCTATTGACAAGTGGTTCTTCATCTCCGGTTCT GTTTAAACATGGAGGCCCAGAATACCC	Forward primer used to create *ipp1-DAmP::natMX4* allele
IPP1	CGCATACGTGGTAGGTGTCTCATTTTCAATTCAA AATATTCAGTATAGCGACCAGCATTCAC	Reverse primer used to create *ipp1-DAmP::natMX4* or *ipp1Δ0::natMX4* allele
IPP1	TAGGTCTATAGAACAGGATATCCCGCCGCGCAAT TTACTAACATGGAGGCCCAGAATACCC	Forward primer used to create *ipp1Δ0::natMX4* allele
RNR2	GCAAGAAGCCGGTGCTTTCACCTTCAACGAAGAC TTTTAACGTACGCTGCAGGTCGAC	Forward primer used to create *rnr2-DAmP::kanMX4* allele
RNR2	TGAAGAGACTGCGTAAAAAGAAATATATAGAGAG ATACTCATCGATGAATTCGAGCTCG	Reverse primer used to create *rnr2-DAmP::kanMX4* or *rnr2Δ0::kanMX4* allele
RNR2	GAATCCAAACTTAATACACGTATTTATTTGTCCA ATTACCCGTACGCTGCAGGTCGAC	Forward primer used to create *rnr2Δ0::kanMX4* allele
RNR2	GCAAGAAGCCGGTGCTTTCACCTTCAACGAAGAC TTTTAAACATGGAGGCCCAGAATACCC	Forward primer used to create *rnr2-DAmP::natMX4* allele
RNR2	TGAAGAGACTGCGTAAAAAGAAATATATAGAGAG ATACTCCAGTATAGCGACCAGCATTCAC	Reverse primer used to create rnr2-*DAmP::natMX4* or *rnr2Δ0::natMX4* allele
RNR2	GAATCCAAACTTAATACACGTATTTATTTGTCCA ATTACCACATGGAGGCCCAGAATACCC	Forward primer used to create *rnr2Δ0::natMX4* allele
ALG7	TATAGGCGCTATCATCTTTGGCCACGACAACCTA TGGACAGTACGTTGACGTACGCTGCAGGTCGAC	Forward primer used to create *alg7-DAmP::kanMX4* allele

ALG7	TGCGTCATAAAAGTACAAAGTAACTACCAATACA TAATCTATCGATGAATTCGAGCTCG	Reverse primer used to create *alg7-DAmP::kanMX4* or *alg7Δ0::kanMX4* allele
ALG7	AGTAGAGCAAGGCGGAGAACGGTAACAAAAAGTA GACTATCGTACGCTGCAGGTCGAC	Forward primer used to create *alg7Δ0::kanMX4* allele
ALG7	TATCATCTTTGGCCACGACAACCTATGGACAGTA CGTTGAACATGGAGGCCCAGAATACCC	Forward primer used to create *alg7-DAmP::natMX4* allele
ALG7	TGCGTCATAAAAGTACAAAGTAACTACCAATACA TAATCTCAGTATAGCGACCAGCATTCAC	Reverse primer used to create *alg7-DAmP::natMX4* or *alg7Δ0::natMX4* allele
ALG7	AGTAGAGCAAGGCGGAGAACGGTAACAAAAAGTA GACTATACATGGAGGCCCAGAATACCC	Forward primer used to create *alg7Δ0::natMX4* allele
ACT1	AAAGGAAATCACCGCTTTGG	Forward primer used in RT-PCR
ACT1	AGATGGACCACTTTCGTCGT	Reverse primer used in RT-PCR
DFR1	GCAACTCCTGCAATGGACACT	Forward primer used in RT-PCR
DFR1	AACCTTTTTCTTCCAGCGAGT	Reverse primer used in RT-PCR
IPP1	TCTCCGGTGAAGCTAAGAACA	Forward primer used in RT-PCR
IPP1	TTGGAGTAGGTTGGGGTGTC	Reverse primer used in RT-PCR
RNR2	GCCTCCATTTTCTGGTTGAA	Forward primer used in RT-PCR
RNR2	TTTCAACAATGGCTGGGTCT	Reverse primer used in RT-PCR
ALG7	CTCCATTTGATTGACCTGGAA	Forward primer used in RT-PCR
ALG7	AAGCCAAAATTCCAATGCAG	Reverse primer used in RT-PCR
DFR1	TGGAGGTCACCAACGTCAAC	Forward junction primer
DFR1	GTTTGACGGCATACCTTGCT	Reverse junction primer
IPP1	CGAAGTTAAGTGCGCAGAAA	Forward junction primer
IPP1	CCAGAACAAAGCAAACAGCA	Reverse junction primer
RNR2	TTTTCGCCTCGACATCATCT	Forward junction primer
RNR2	TGAGATTGCCTTTGCTGTTG	Reverse junction primer
ALG7	TTTTAATCAAATGTTAGCGTGATTT	Forward junction primer
ALG7	CCGGTTATCTTGACCTCGGTA	Reverse junction primer
DFR1	ACGCTACTCGCTGGAAGAAA	Forward cassette primer
DFR1	TACTGCAGGTGAGGCTGAAA	Reverse cassette primer
IPP1	TGGTTCTTCATCTCCGGTTC	Forward cassette primer
IPP1	GGCCTTGTAGGAGGCAAGAT	Reverse cassette primer
ALG7	TGCATTGGAATTTTGGCTTT	Forward cassette primer
ALG7	ACGAAGTAAAGGGCTGGACA	Reverse cassette primer
RNR2	GTCGAAAACCCCTTCGATTT	Forward cassette primer
RNR2	GCGAAAGCCCACATAAAGAG	Reverse cassette primer