A Noncomplementation Screen for Quantitative Trait Alleles in *Saccharomyces cerevisiae*

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ABSTRACT Both linkage and linkage disequilibrium mapping provide well-defined approaches to mapping quantitative trait alleles. However, alleles of small effect are particularly difficult to refine to individual genes and causative mutations. Quantitative noncomplementation provides a means of directly testing individual genes for quantitative trait alleles in a fixed genetic background. Here, we implement a genome-wide noncomplementation screen for quantitative trait alleles that affect colony color or size by using the yeast deletion collection. As proof of principle, we find a previously known allele of *CYS4* that affects colony color and a novel allele of *CTT1* that affects resistance to hydrogen peroxide. To screen nearly 4700 genes in nine diverse yeast strains, we developed a high-throughput robotic plating assay to quantify colony color and size. Although we found hundreds of candidate alleles, reciprocal hemizygosity analysis of a select subset revealed that many of the candidates were false positives, in part the result of background-dependent haploinsufficiency or second-site mutations within the yeast deletion collection. Our results highlight the difficulty of identifying small-effect alleles but support the use of noncomplementation as a rapid means of identifying quantitative trait alleles of large effect.

KEYWORDS
complex trait
copper sulfate
hydrogen sulfide

Identifying genes responsible for phenotypic variation in natural populations is difficult because most traits are influenced by multiple genes and because the effects of each gene must be mapped within a heterogeneous genetic background. Both linkage mapping and genome-wide association studies overcome this heterogeneity by measuring the average effect of a gene over a large number of samples. However, the two approaches detect qualitatively different types of alleles. Linkage mapping often reveals alleles with large and in some cases epistatic effects that are rare in the general population (*e.g.*, Deutschbauer and Davis 2005; Ben-Ari *et al.* 2006; Sinha *et al.* 2006; Gerke *et al.* 2009). In contrast, genome-wide association studies often identify small-effect associations with common alleles and find little evidence of epistasis (Altshuler *et al.* 2008). Although many factors likely contribute to these differences (*e.g.*, Gerke *et al.* 2010), our understanding of quantitative trait alleles depends on both how they are mapped and our ability to map them (Rockman 2012).

One particularly undersampled source of variation is rare alleles of moderate or small effect (Pritchard 2001; Wang *et al.* 2005). Under a rare alleles model, alleles segregating in one cross are expected to be absent in other crosses because they are rare in the general population. Furthermore, most rare alleles are not detected by population association because power is a function of allele frequency. The larger number of rare missense or nonsense alleles in case compared with control samples supports the contribution of rare alleles to a number of complex human genetic diseases (*e.g.*, Cohen *et al.* 2004; Fearnhead *et al.* 2004; Ahituv *et al.* 2007). However, without a population-based screen for quantitative trait alleles that does not depend on their frequency, the amount of variation explained by rare alleles has been difficult to assess.

Quantitative noncomplementation provides a means of identifying and measuring the effect of an allele. The idea is that the effect of a recessive or partially recessive allele will be revealed in the absence of a wild-type allele, whereas the effect of a dominant allele, typically wild type, will remain unchanged (Figure 1). Quantitative noncomplementation has been predominantly used to fine-map quantitative trait loci (Mackay 2004). However, it can also be used to screen the genome when a large number of mutations are available (*e.g.*, Coyne *et al.* 1998; Takahashi *et al.* 2011). In the context of a genome-wide screen, quantitative noncomplementation offers two distinct advantages over linkage and association studies. First, it can be applied to multiple

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genomes, making it independent of allele frequency. Second, a fixed background can be used to make it more sensitive to alleles with small or epistatic effects, even when large-effect alleles are also present at other loci. However, quantitative differences in noncomplementation can also result from background-dependent dominance of the mutation being complemented (Service 2004). In yeast, the reciprocal hemizygosity test (Steinmetz *et al.* 2002) has been extensively used to control for any interactions with genetic background, including background-dependent dominance, by comparing two hybrid strains that only differ by the allele present at a single hemizygous locus (Figure 1).

In this study, we used the *Saccharomyces cerevisiae* deletion collection (Giaever *et al.* 2002) to conduct a genome-wide noncomplementation screen for quantitative trait alleles underlying two copper-related traits. We mated nine diverse strains to both the *MAT*a and *MAT*α deletion collections and compared these with two control crosses involving the parents of the deletion collection. To control for interactions with the genetic background a subset of noncomplementing, candidates were tested for quantitative trait alleles by reciprocal hemizygosity analysis. Although we recovered a known mutation of large effect in *CYS4* (Kim and Fay 2007), as well as a novel allele of *CTT1* that confers sensitivity to hydrogen peroxide, most of the candidate quantitative trait alleles identified in our screen appear to be false positives related to background-dependent dominance of the deletion or second-site mutations within the yeast deletion collection.

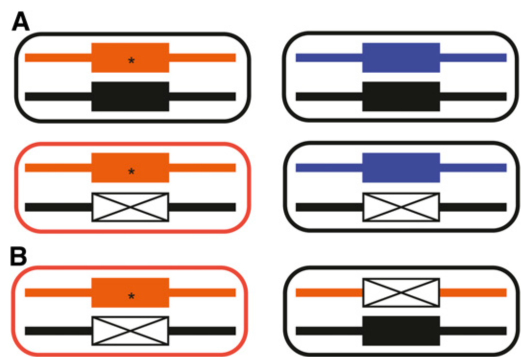

Figure 1 Identification of quantitative trait alleles via quantitative noncomplementation and reciprocal hemizygosity analysis. (A) Noncomplementation is shown by a recessive quantitative trait allele (*) that has a different effect in the hemizygous (bottom left) compared with the heterozygous (top left) state. A wild-type allele (blue) is expected to have the same effect in both states (right), assuming no haploinsufficiency. Colors indicate two wild strain alleles (orange and blue) and a laboratory strain allele (black). Rounded rectangles show strains with different phenotypes (orange *vs.* black). Gene deletions are indicated by a cross within a white background. (B) A reciprocal hemizygosity test reveals a quantitative trait allele by differences between two reciprocal hemizygotes. No differences are expected as a result of interactions between the dominance of the deletion (haploinsufficiency) and the hybrid background because both hemizygotes have a fixed genetic background except at the locus of interest.

MATERIALS AND METHODS

Strains and media

Rich medium (2% yeast extract, 1% peptone, 2% dextrose, 2% agar), G418 medium (rich medium, 200 mg/L G418), hydrogen peroxide medium (rich medium, 0.0038% hydrogen peroxide), copper sulfate medium (rich medium, 3 mM $CuSO_4$), and PPG medium (rich medium, 1 mM $CuSO_4$, 320 μM propargylglycine; PPG) were prepared at a volume of 50 mL/plate and dried for 12 hr at room temperature.

Heterothallic strains were generated by deleting *HO* using *dsdAMX* (Vorachek-Warren and McCusker 2004), *natMX* (Goldstein and McCusker 1999) for BC187, dissecting tetrads and selecting *MAT*a or *MAT*α haploids. Only *MAT*α haploids were obtained for YJM210, DBVPG1106, and UWOPS87 and only *MAT*a for Y12. For hybrid selection, *TRP1* was deleted using *hghMX* (Goldstein and McCusker 1999) and *natMX* for UWOPS87. For control matings, *TRP1* was deleted using *kanMX* in BY4741 (*MAT*a, *his3*Δ*1*, *leu2*Δ*0*, *met15*Δ*0*, *ura3*Δ*0*) and BY4742 (*MAT*α, *his3*Δ*1*, *leu2*Δ*0*, *lys2*Δ*0*, *ura3*Δ*0*). Yeast deletion collections within the BY4741 (*MAT*a, 4695 deletions), BY4742 (*MAT*α, 4680 deletions), and BY4743 (*MAT*a/*MAT*α *his3*Δ*1*/*his3*Δ*1* *leu2*Δ*0*/*leu2*Δ*0* *LYS2*/*lys2*Δ*0* *met15*Δ*0*/*MET15* *ura3*Δ*0*/*ura3*Δ*0*, 4670 deletions) background were provided by Linda Riles and Mark Johnston (Washington University). YJF173 (S288c derivative, *MAT*a, *ho*-, *ura3*-*52*) and other strains backgrounds used in the reciprocal hemizygosity test are described in supporting information, Table S1.

Hemizygous deletion collections

Five of the nine haploid strains were crossed to both the *MAT*a and *MAT*α deletion collections. Two control strains (BY4741 *trp1*Δ::*kanMX* and BY4742 *trp1*Δ::*kanMX*) were also mated with the deletion collection of opposite mating type. For each mating, the haploid strain was spotted on rich medium plates (384 strains/plate), the deletion collection was spotted on top using a Singer Rotor robot (Singer Instruments, Somerset, UK), and plates were incubated for 24 hr at 30°. Colonies, a mixture of hybrid diploid and haploids, were replica plated onto medium lacking tryptophan, uracil, leucine, and lysine. To test the fraction of diploid cells from a colony on selection medium, colonies were suspended in water, plated on rich medium, and 100 single colonies were transferred to selective media. For matings with two test deletions, *MET10* and *CYS4*, all the colonies tested were diploid. In addition, hemizygosity was confirmed by polymerase chain reaction (PCR) amplification of two different-sized fragments at *MET10* in all 14 hemizygous strain collections.

Phenotyping

A set of ~4.7K diploid hybrids for each of the 14 natural isolates (10 representing opposite mating types of five distinct strains), two heterozygous deletion controls, and the homozygous deletion collection were replica-plated onto rich medium, copper sulfate, and PPG medium and grown for 48 hr at 30°, a total of 240K colonies. Digital photographs were taken at 24 hr for copper sulfate sensitivity and 48 hr for colony color. Images were trimmed, and red and green channel images were saved using Photoshop (Adobe, San Jose, CA). Spotfinder (Saeed *et al.* 2003) was used to grid the colonies and measure colony size and color. The red channel was used to grid the colonies and measure colony size using the Otsu method, and colony color was measured using the green color intensity. To control for edge effects (colony color is darker at the edges of a plate), raw color values were divided by median color of the column or row depending on which one generated values closest to the overall median. Colony size on copper sulfate medium was divided by that on rich medium to control for copper independent growth differences. Each collection of strain phenotypes was divided by its median. Candidate genes were selected by those that passed a cutoff of less than 0.8 or greater than 1.2 for color and less than 0.8 or greater than 1.4 for size. These cutoffs represent phenotypes that were just large enough to see. Candidate genes were subsequently eliminated if the hemizygous controls showed effects less than 0.95 or greater than 1.05.

Phenotype assays were replicated for 36 candidate genes that passed the same cutoffs in the homozygous deletion collection. The diploid hemizygotes were regenerated, and phenotypic differences between Strain/BYΔ and Strain/BY were compared with the difference between the controls, BY/BYΔ and BY/BY, using analysis of variance where Strain indicates one of the natural strains, BY indicates one of the parents of the deletion collection, and BYΔ indicates a deletion strain.

Interactions with genetic background

A small set of genes were further examined for differences between hemizygotes generated using the BY4741 (*MAT*a) and BY4742 (*MAT*α) deletion collections. First, the hemizygotes were tested for loss of heterozygosity at *LYS2* and *MET15* for nine candidate genes (*SAM2*, *ATG17*, *DBP7*, *UBI4*, *RAV2*, *MRPL22*, *MOT2*, *RIM11*, and *COQ10*) that showed effects in the BY4741 or BY4742 hemizygotes but not both. No loss of heterozygosity was found. Second, the effect of the *MET15*, *LYS2*, and *MAT* genotype was tested for five of the genes (*SAM2*, *UBI4*, *RAV2*, *MRPL22*, and *COQ10*). Homozygous diploid deletion strains (BY4741/BY4742) were sporulated, tetrad were dissected, and one or two spores from each of the four possible progeny, *lys2*-/*met15*-, *LYS2*/*MET15*, *LYS2*/*met15*-, and *lys2*-/*MET15*, were obtained for both mating types and mated to M22 (*SAM2*, *UBI4*), YPS163 (*RAV2*, *MRPL22*), or YJM326 (*COQ10*). A significant difference in noncomplementation among hemizygotes with different genetic backgrounds was tested by analysis of variance using the model: $y_i = \mu + MAT + MET + LYS + MAT^*MET + MAT^*LYS + MET^*LYS + \varepsilon_i$, where y_i is the phenotype of strain i; μ is the average phenotype across all strains; *MAT*, *MET* and *LYS* are the effects of the *MAT* locus, *MET15* deletion, and *LYS2* deletion, respectively, and ε_i is the error.

Reciprocal hemizygosity analysis

Deletions were generated for 18 of 25 candidate genes in each haploid strain as well as the progenitor of the deletion collection. Seven of the genes were not tested because of difficulties in generating either one or both deletions required for the reciprocal hemizygosity test. Deletions were generated by PCR amplification of the *kanMX* deletion cassette and lithium acetate transformation (Wach *et al.* 1994) and were confirmed by PCR. Two or more independent deletions were generated for each gene in each strain except for deletions of *RBK1* in CLIB382 and Y12, *SIN3* in Y12, *BUL1* in BC187, and *BUD31* in UWOPS87, where only one independent deletion was generated. Strains with only one deletion (except *BUL1*) and strains showing inconsistent phenotypes between independent deletions, including deletions from the yeast deletion collection, were tested for linkage between the phenotype and the deletion by mating the deletion strain to the progenitor of the deletion collection, sporulating the diploids, dissecting three or more tetrads, and phenotyping. For an unlinked second-site mutation, the probability of cosegregation of G418 resistance and colony color or size in three tetrads is $(1/6)^3$ or 0.005. Using this method, we found *TUS1* and *RBK1* to have effects caused by second-site mutations within the yeast deletion collection, and *YBR300C* was found to have effects caused by a second-site mutation within the newly generated *YBR300C* deletion strain.

Phenotype differences between reciprocal hemizygotes were tested by analysis of variance using the model: $y_i = \mu + H + R + \varepsilon_i$, where y_i is the phenotype of strain i, μ is the average phenotype across all strains, H is the type of hemizygote (Strain/BYΔ or StrainΔ/BY), R is for replicates obtained from independent deletions, and ε_i is the error.

Replication of reciprocal hemizygosity analysis

Gene deletions were generated as described previously, except that laboratory strain deletions were made in YJF173 rather than BY4741 or BY4742. Deletions were backcrossed to progenitors of the opposite mating type, progeny sporulated, and haploids of the desired genotype were selected by PCR of the mating type locus and assaying resistance to G418 as an indicator of the deletion. Segregants that did not exhibit a consistent deletion phenotype were backcrossed until phenotypic homogeneity was achieved. *FRA1* and *BUD31* from UWOPS87 were not included because of our inability to obtain 2:2 segregation of the deletion in the backcrossed progeny. Further problems arose when we remade the *FRA1* and *BUD31* deletions in heterothallic diploid versions of UWOPS87 and found that the resulting monosporic clones were diploid at the mating type locus. For *GDE1* we measured both colony color and size because it was identified in the size screen but showed color effects in the initial reciprocal hemizygosity analysis. However, *GDE1* alleles did not affect colony color (see *Results*) or size (not shown). For *MRPL22* in BC187, we only measured effects in YJF173 *MAT*a x BC187 *MAT*α hybrids because we were not able to recover both deletions of the opposite mating types.

Colony color and size were measured for each strain using the average of four colonies on the same plate and from two pictures of the same plate, one in the forward and one in the reverse direction. The position of each strain was randomized across the plate excluding border positions filled by YJF173. Pictures in two orientations were taken to control for a subtle trend in colony color across the plate as the result of variation in the amount of light reflected by each colony. Significant differences between reciprocal hemizygotes were tested using a linear model $y_i = \mu + H + M + \varepsilon_i$, where y_i is the phenotype of strain i, μ is the average phenotype across all strains, H is the type of hemizygote, M is the cross direction (Lab *MAT*a x Wild *MAT*α or Wild *MAT*a x Lab *MAT*α), and ε_i is the error. If mating type was not significant, the term was dropped from the statistical analysis. To determine whether the significance of *DUG3* and *CTT1* alleles depends on the assumptions of the analysis of variance we also used a Wilcoxon rank sum test and found significant results for both (P = 0.0062 and P = 0.014, respectively). Haploinsufficiency was tested using an analysis of variance term to distinguish the two hemizygous hybrids from the hybrid without a deletion.

Statistics

Unless otherwise noted, all P values were generated by fitting linear models using the "lm" package of R (http://www.R-project.org) and testing for significant terms using analysis of variance.

RESULTS

Identification of *CYS4* and *CTT1* quantitative trait alleles

To evaluate the capability of a quantitative noncomplementation screen to identify quantitative trait alleles we examined colony color in M22. M22 colonies are rust-colored in the presence of copper sulfate because of a recessive, loss of function, nonsynonymous mutation in cystathionine beta-synthase (*CYS4*) (Kim and Fay 2007; Kim *et al.* 2009). We mated M22 to the yeast deletion collection and examined colony color of the resulting collection of hemizygous strains. Only a single strain, hemizygous for *CYS4*, showed a noticeable difference in colony color (Figure 2).

To further evaluate a quantitative noncomplementation screen, we examined sensitivity to hydrogen peroxide in YPS163. YPS163 is particularly sensitivity to hydrogen peroxide, but no genes have yet been found to be responsible for this sensitivity (Kim and Fay 2007).

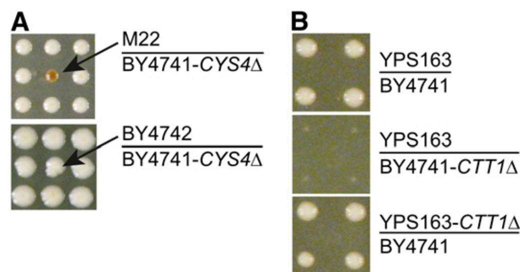

Figure 2 Identification of quantitative trait alleles of *CYS4* and *CTT1*. (A) A noncomplementation screen of M22/BY4741 hemizygotes identifies the M22 allele of *CYS4* as a colony color allele. Hemizygotes were generated by crossing M22 and BY4742 (control) to the BY4741 *MAT*a deletion collection, and colony color was assayed on copper sulfate medium. The *CYS4* hemizygote of M22 (M22/BY4741-*CYS4*Δ) and BY4742 (BY4742/BY4741-*CYS4*Δ) are shown along with strains at adjacent positions on the plate, which are hemizygous for different genes. (B) Reciprocal hemizygosity analysis shows that the YPS163 allele of *CTT1* affects sensitivity to hydrogen peroxide. The YPS163 allele of *CTT1* does not complement a *CTT1* deletion (YPS163/BY4741-*CTT1*Δ), whereas the BY4741 allele of *CTT1* does complement (YPS163-*CTT1*Δ/BY4741). Each photograph shows four replicate colonies of each strain.

We mated YPS163 to the yeast deletion collection and found that only the strain hemizygous for *CTT1* showed sensitivity to hydrogen peroxide similar to that of YPS163. *CTT1* encodes a cytosolic catalase that protects cells from oxidative damage by converting hydrogen peroxide to water and oxygen (Grant *et al.* 1998). *CTT1* was deleted in YPS163 and BY4741, and the resulting strains were used to obtain two reciprocal hemizygotes, each carrying a different allele of *CTT1*. The two hemizygotes showed allele-specific complementation; only the YPS163 allele of *CTT1* failed to complement the *CTT1* deletion (Figure 2). The results of these two preliminary screens motivated us to conduct a more comprehensive screen for quantitative trait alleles by crossing multiple strains to the yeast deletion collection and testing each hemizygote for non-complementation.

Large-scale screen for colony color and size alleles

To scale the noncomplementation screen to multiple strains, we examined two traits that vary among nine diverse yeast strains, colony size in the presence of copper sulfate and colony color in the presence of copper sulfate and PPG (Figure 3). PPG is an inhibitor of cystathionine gamma-lyase (*CYS3*) and causes typically white-colored strains to become rust-colored in the presence of copper sulfate (Kim *et al.* 2009). Both traits may have ecological significance: rust coloration is dependent on hydrogen sulfide production (Kim *et al.* 2009), a significant problem in wine production (Linderholm *et al.* 2008), and resistance to copper sulfate has been hypothesized to be an adaptation to fungicides sprayed in vineyards (Mortimer 2000). The nine strains were selected on the basis of genome sequencing projects (Doniger *et al.* 2008; Liti *et al.* 2009) and their phenotypic diversity within a larger collection of yeast strains (Figure S1).

For each of the nine strains, we generated heterothallic derivatives with a dominant selectable marker at the *TRP1* locus for mating. Five of strains (M22, YPS163, YJM326, BC187, and CLIB382) were mated to both the *MAT*a (BY4741) and *MAT*α (BY4742) deletion collections, and the other four were mated to only one. As a control, we also mated the parents of the deletion collection to the *MAT*a and *MAT*α deletion collections. Including the controls, the ~75K hemizygotes

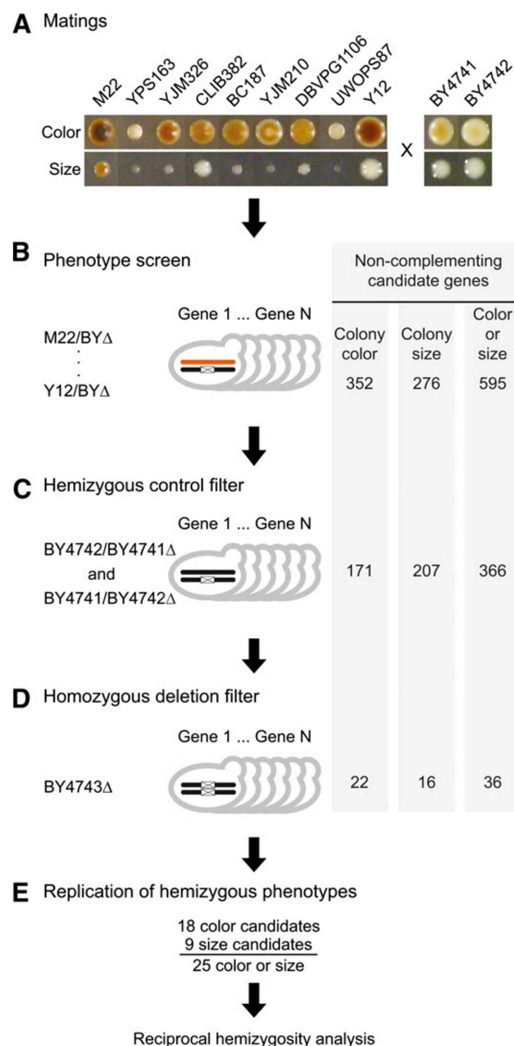

Figure 3 Overview of noncomplementation screen for colony color and size alleles. (A) Colony color (PPG medium) and size (copper sulfate medium) is shown for strains crossed to the *MAT*a (BY4741) and *MAT*α (BY4742) deletion collections. Colony color and size of the BY4741 and BY4742 parental strains are shown as representatives. (B) The resulting collections of hemizygotes were phenotyped for colony color and size (see *Materials and Methods*). BYΔ indicates either BY4741 or BY4742 deletion strains. (C) Control matings of BY4742 (parent) to BY4741Δ (deletions) and the reciprocal set of crosses were used to eliminate candidates that exhibited haploinsufficiency. (D) Candidates that showed no effect in the homozygous deletion collection (BY4743Δ) also were eliminated. (E) Replicated measurements of colony color and size were obtained for manually generated hemizygotes. Reciprocal hemizygosity analysis was used to test the remaining candidates for quantitative trait alleles. The number of noncomplementing candidate genes indicates the remaining number after each filter.

were phenotyped by robotic arraying of the strains in a 384-colony format and measuring colony color on PPG medium and colony size on copper sulfate medium using semi-automated image analysis (see *Materials and Methods*).

A total of 595 genes showed noncomplementation for colony color or size in one or more strains (Figure 3). After eliminating genes that showed effects in the hemizygous controls, we found 366 noncomplementing candidates, an average of 25 and 39 genes per strain, for

colony color and size, respectively (Table S2 and Table S3). Most of the genes, 65%, were only identified in one of the nine strains (Figure S2).

Interactions with genetic background

Many of the noncomplementing candidate genes showed different effects in hemizygotes generated using the *MAT*a *vs.* the *MAT*α deletion collection (Figure 4). Of the 268 genes that were identified in the five strains crossed to both the *MAT*a and *MAT*α deletion collection, only 19 were identified in both crosses. Some of these differences are likely due to measurement error and the cutoffs used to identify noncomplementing genes. However, differences could also result through interactions with the genetic background.

Strains generated using the *MAT*a and *MAT*α deletion collection differ for two auxotrophic markers, *MET15* and *LYS2*, origin of mating type, and any second-site (unintentional) mutations that occurred during the construction of the deletions. To test whether the *MET15* deletion, *LYS2* deletion, or the mating type locus could explain the difference between hemizygotes generated using the *MAT*a and *MAT*α deletion collection, we tested five genes with a large effect in one hemizygote but not the other. Differences in the genetic background were assorted using recombinant strains generated by sporulating a diploid hybrid of the two haploid deletion strains from the *MAT*a and *MAT*α deletion collection. Using hemizygotes generated with the recombinant strains, we found noncomplementation of two genes, *SAM2* ($P = 2.2 \times 10^{-16}$) and *UBI4* ($P = 6.3 \times 10^{-16}$), was dependent on the *LYS2* deletion. For the remaining three genes, phenotypic variation among the hemizygotes was not associated with mating type or auxotrophies, raising the possibility that second-site mutations were present in one or both of the deletion strains. Regardless of the cause, our results suggest that auxotrophic markers and second-site mutations are not entirely recessive as is often assumed (Grünenfelder and Winzeler 2002).

Reciprocal hemizygosity analysis

Candidate genes may be false positives because of the dominance of the deletion (haploinsufficiency) or dominance of second-site mutations within the yeast deletion collection. Although both the deletion and any second-site mutations were also present in the hemizygous controls, their effects may depend on the hybrid background. We used reciprocal hemizygosity analysis (Steinmetz *et al.* 2002) to account for this possibility.

Two filters were used to select noncomplementing candidates for reciprocal hemizygosity analysis. First, we selected those candidate genes that showed a significant effect in the homozygous diploid deletion collection. Interestingly, only 22 of 171 and 16 of 207 candidate genes showed phenotypic effects in the homozygous deletion collection for colony color and size, respectively. Candidate genes without effects in the homozygous deletion collection could be false positives but could also arise as the result of differences between the genetic background of the hybrid hemizygotes and the homozygous deletion collection. Second, we retested the resulting 36 candidate for noncomplementation by manually regenerating each hemizygous strain and obtaining replicated phenotype measurements. After replication, 25 of 36 candidate genes retained evidence of noncomplementation (Figure 3 and Table S4). We were able to generate deletions in both the laboratory and wild strain(s) for 18 of the remaining 25 candidate genes. Because some genes were identified in more than one strain, we generated deletions that enabled us to test a total of 44 candidate alleles by reciprocal hemizygosity analysis.

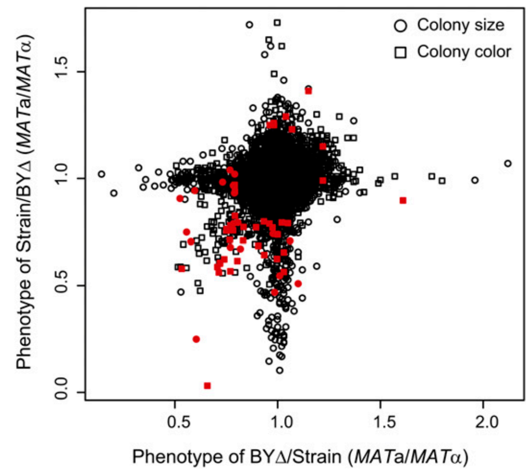

Figure 4 Noncomplementation phenotypes differ between the *MAT*a and *MAT*α deletions collections. Colony size (circles) and colony color (squares) phenotypes are shown for hemizygotes generated using both the *MAT*a and *MAT*α deletion collections (M22, YPS163, YJM326, BC187, and CLIB382). Noncomplementing candidates are shown in red and are defined by genes that exhibit an effect in one or more of the wild strain hemizygotes but not the hemizygous controls (BY4741 and BY4742 matings), and an effect in the homozygous deletion control (BY4743). Phenotypes are normalized to one by the mean of each hemizygous deletion collection (see *Materials and Methods*).

Of the 44 alleles in 18 genes that were tested, 14 alleles in 10 genes showed significant differences between the two reciprocal hemizygotes (Table S5). For one of these, *GDE1*, we found effects on colony color even though it originally only passed our screening filters for colony size. For the eight candidate genes that were not validated by reciprocal hemizygosity: *YGL165C* was discarded because it is a dubious gene and overlaps with another candidate, *CUP2*; *SNF7*, showed evidence for haploinsufficiency; three genes (*RBK1*, *TUS1*, and *YBR300C*) were false positives as the result of second-site mutations; and three genes had deletion phenotypes inconsistent with the deletion collection (*ATG15*, *MAL31*, and *YCR087W*).

Of the 14 alleles identified by reciprocal hemizygosity analysis, most showed small, barely noticeable effects, much smaller than the effects of the haploid deletions (Figure S3 and Table S5). However, the effects of the haploid deletions were often smaller in the wild strain than in the laboratory strain, consistent with a recessive loss of function mutation in the wild strain. We sequenced all 14 alleles and found two of the alleles, *ATG17* in M22 and *BUL1* in BC187, had no mutations within either the coding or adjacent noncoding regions in comparison with the laboratory strain. Although we generated multiple independent deletions or observed cosegregation between the deletion and the phenotype of interest, the sequence analysis raised the possibility that second-site mutations occur commonly enough to generate small but consistent phenotype differences between the reciprocal hemizygotes. As such, we decided to replicate the reciprocal hemizygosity analysis using deletion strains that were backcrossed to their progenitor to eliminate any potential second-site mutations generated during transformation.

Replication of reciprocal hemizygosity analysis

To replicate the reciprocal hemizygosity test, we independently derived new deletions for each gene, backcrossed these strains to their parent, and selected 10 segregants with the deletion from each

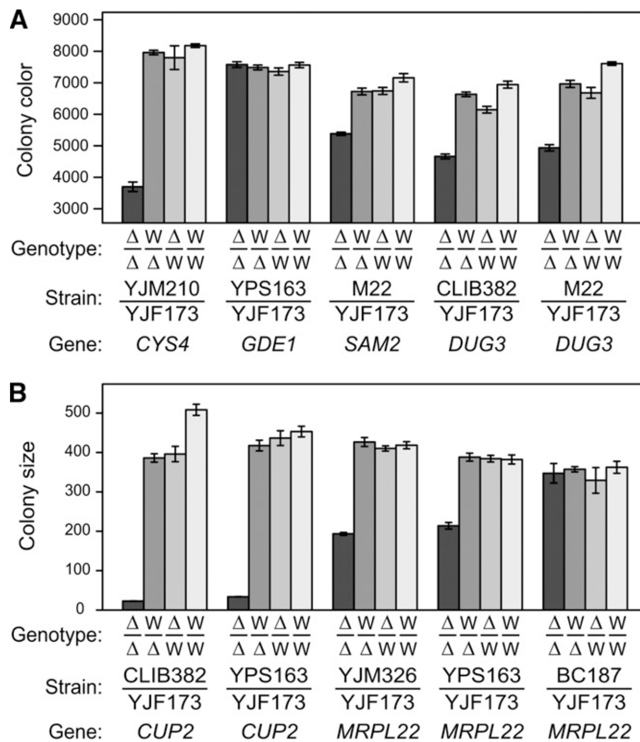

Figure 5 Replication of reciprocal hemizygosity analysis for colony color and size alleles. (A) Colony color. (B) Colony size. The mean and standard error (bars) for four genotypes are shown for a hybrid between a laboratory strain (YJF173) and another strain (middle labels). The homozygous deletion (Δ/Δ), laboratory allele deletion (W/Δ), wild allele deletion (Δ/W), and no deletion (W/W) of the gene being tested (bottom labels), where Δ indicates a deletion and W indicates wild-type allele present in the strain.

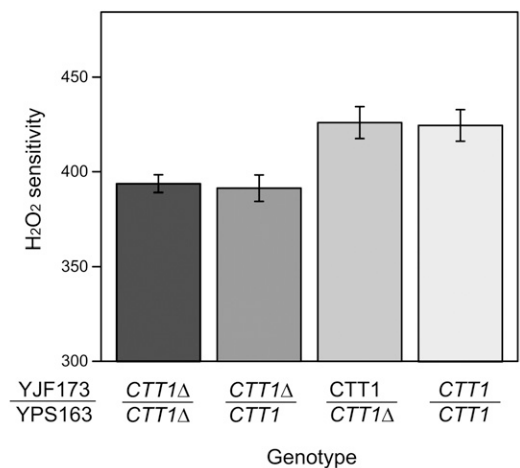

Figure 6 Replication of reciprocal hemizygosity analysis for *CTT1* and sensitivity to hydrogen peroxide. The mean and standard error (bars) is shown for four hybrids (YJF173/YPS163) with genotypes at *CTT1* labeled on the bottom, (Δ = deletion). Sensitivity to hydrogen peroxide was measured by colony size.

backcross for reciprocal hemizygosity analysis, five of each mating type. Rather than using the parent of the yeast deletion collection, which has a number of auxotrophies, we used a different version of S288c with only a single auxotrophy (YJF173, Table S1). *CTT1* was also included in the analysis because our previous reciprocal hemizygosity analysis showed large phenotypic differences and we wanted to ensure these differences can be attributed to *CTT1*. However, we excluded the two genes without mutations, *ATG17* in M22 and *BUL1* in BC187, and two genes for which we were not able to obtain backcross progeny, *FRA1* and *BUD31* in UWOPS87.

Out of the 11 alleles tested, three showed significant differences between reciprocal hemizygotes that depended on the cross direction, defined by either a *MAT*a or *MAT*α strain mated to the laboratory strain of the opposite mating type (Figure 5 and Figure S4). For *CYS4*, the dependency on the cross direction could be the result of differences between the YJM210 *MAT*a and *MAT*α strain; strains generated with YJM210 *MAT*a were consistently darker than those generated with YJM210 *MAT*α (Figure S4). For *SAM2*, the two cross directions generated hemizygous effects in opposite directions (Figure S4). For *DUG3* in M22, one cross direction showed an effect consistent with DUG3 in CLIB382 and the other showed no effect.

Two of the 11 alleles showed significant differences between reciprocal hemizygotes that did not depend on the cross direction (Figure 5); *DUG3* in CLIB382 (P = 0.0024, Figure 5) and *CTT1* in YPS163 (P = 0.0054, Figure 6). The difference between the two *DUG3* alleles is 21% of the difference between the homozygous deletion (*DUG3*Δ/*DUG3*Δ) and wild-type (*DUG3*/*DUG3*) hybrid and 13% of the difference between the two haploid parent strains without the *DUG3* deletion, CLIB382 and YJF173. However, the *DUG3* hemizygotes generated using YJF173 had phenotypes that were inconsistent with the initial reciprocal hemizygotes generated using BY4741. Hybrids with a hemizygous YJF173 allele were darker than those with a CLIB382 allele of *DUG3*, indicating that the YJF173 allele confers a darker colony color, rather than a lighter color as originally found (Figure S3 and Table S5). We observed a similar but insignificant trend for *DUG3* in M22/YFJ173 hemizygotes (Figure 5 and Figure S4). Finally, we found the YPS163 allele of *CTT1* in the hemizygous state has a large effect on sensitivity to hydrogen peroxide, equivalent to a deletion (Figure 6), and consistent with our previous reciprocal hemizygosity analysis.

DISCUSSION

Identifying quantitative trait alleles depends on our ability to map and resolve these alleles to individual genes. However, both linkage and linkage disequilibrium mapping require large sample sizes to detect the effects of a locus across a heterogeneous genetic background. Thus, many quantitative trait alleles may be missed if they only have effects in certain backgrounds (Li *et al.* 2005; Sinha *et al.* 2006; Kim and Fay 2007; Dowell *et al.* 2010). To compensate for this deficiency, we implemented a genome-wide noncomplementation screen for quantitative trait alleles using the yeast deletion collection. Paradoxically, we show that most of the candidate quantitative trait alleles we identified are likely false-positive results because of subtle genetic interactions between the locus of interest and the near-isogenic background. Compared with the identification of two large-effect quantitative trait alleles of *CYS4* and *CTT1*, our results highlight the crux of investigating quantitative trait alleles with small and/or background-dependent effects.

Using the noncomplementation screen, we were able to identify two large-effect quantitative trait alleles. The M22 allele of *CYS4* was used as a positive control as it is recessive and causes an easily measured effect on colony color, nearly equivalent to that of a null allele (Kim *et al.* 2009). We also found a novel allele of *CTT1* in YPS163 that causes sensitivity to hydrogen peroxide. In a previous study,

Diezmann and Dietrich (2011) found no effects of *CTT1* on sensitivity to hydrogen peroxide. However, they examined linkage in a cross between a laboratory strain, S288c, and a clinical isolate, YJM789, and YPS163 has four candidate amino acid polymorphisms in *CTT1* not present in either S288c or YJM789 (F212Y, A255E, D431N, T522A).

Many of the candidates identified in the initial noncomplementation screen were false positives. Although our high-throughput phenotyping assay was quite reproducible when applied to the same hybrid strains, we found 31% (11/36) of candidate alleles did not show replicable effects when hybrids were manually generated and tested for quantitative noncomplementation. Less definitive but still informative, we found only 7% (19/268) of candidates were identified using both the *MAT*a and *MAT*α deletion collection. Out these 19, 10 showed effects in the homozygous deletion collection, and four (*CUP2*, *DUG3*, *ATG15*, and *BUL1*) of these were positive within our initial reciprocal hemizygosity test. Thus, there is no apparent benefit of only using candidates identified in both the *MAT*a and *MAT*α deletion collection, although these two collections were generated independently of one another.

One source of false positives is likely the deletion collection itself. Within the yeast deletion collection, 8% of strains are estimated to carry chromosomal aneuploidies (Hughes *et al.* 2000), and during the construction of the deletion collection, 6.5% of strains exhibited an overt phenotype that did not segregate with targeted deletion (Grünenfelder and Winzeler 2002), implying the presence of many other second-site mutations without an overt phenotype. Although we considered only investigating candidates with effects in both collections, we did not use both collections for all nine strains, and some of the differences between the two collections could be true positives that depend on auxotrophic differences. In particular, BY4741 carries a *MET15* deletion and *MET15* affects colony color (Kim *et al.* 2009). Instead, crosses between BY4741 and BY4742 deletion strains showed that noncomplementation depended on the *LYS2* deletion for two of five genes tested. Thus, at least some of the differences between the two deletion collections can be attributed to allele differences that depend on the genetic background, as has been found in other quantitative genetic studies (Perlstein *et al.* 2007; Kim and Fay 2007).

Another potential indicator of false positives was that only 10% (36/366) of the noncomplementing genes showed effects in the homozygous deletion collection. Although even essential genes can be nonessential in other strain backgrounds (Dowell *et al.* 2010), we decided to only apply the reciprocal hemizygosity test to the subset of candidates that also exhibited effects in the homozygous deletion collection. However, it is possible that some of the candidates that were not tested are true positives.

We used reciprocal hemizygosity analysis as the primary means of eliminating false positives caused by to haploinsufficiency, second-site mutations within the yeast deletion collection, or other unknown causes. Using this approach we eliminated 8 of 18 candidate genes, a total of 30 of 44 candidate alleles. However, not all false positives were eliminated; two of the genes contained no mutations that could underlie the allele differences. One potential explanation is that second site mutations with subtle effects were frequently generated during transformation. Although we attempted to account for this possibility by generating at least two reciprocal hemizygotes using independently generated deletions, second-site mutations may be positively selected to compensate for the deletion, or they may tend to generate phenotypic effects in a consistent direction.

To more carefully control for the effects of second-site mutations, we replicated the reciprocal hemizygosity test using deletion strains that were backcrossed to their parents. However, a number of observations obscure definitive conclusions on the presence or absence of quantitative trait alleles in these genes. Although we found that the *DUG3* allele from YJF173 conferred a darker colony color than the CLIB382 allele, this effect was opposite of that observed in our original reciprocal hemizygosity analysis. The *DUG3* allele of YJF173 also conferred a lighter color than that from M22, but this difference depended on the direction of the cross. Two other genes, *CYS4* and *SAM2*, showed hemizygous effects that depended on the cross direction. Interestingly, *CYS4* in YJM210 carries an amino acid mutation, S504N, that has been reported to affect fermentation rate and hydrogen sulfide production in a vineyard strain (Linderholm *et al.* 2006). Thus, there is evidence both for and against the presence of small-effect quantitative trait alleles in these genes. One additional factor that may be relevant is that four of the hybrids showed evidence of haploinsufficiency, *SAM2* in M22 ($P = 0.005$), *DUG3* in CLIB382 ($P = 5.8 \times 10^{-4}$), *DUG3* in M22 ($P = 1.5 \times 10^{-5}$), and *CUP2* in CLIB382 ($P = 1.1 \times 10^{-6}$). Haploinsufficiency may make these genes particularly susceptible to subtle differences in the genetic background.

The noncomplementation screen is expected to miss certain quantitative trait alleles. Any alleles of essential genes would be missed because we only tested the ~4,700 nonessential genes within the haploid yeast deletion collection. Certain colony color alleles could have been missed if colony size were small as the result of simultaneous effects on copper sensitivity. Although this may have occurred in a few instances, the hybrids used for the colony color screen were fairly uniform in size. A potentially large source of false negatives was our stringent filter to eliminate any genes showing evidence of noncomplementation in the control crosses generated using the parents of the deletion collection. When sensitive phenotyping is used, most deletions exhibit at least some degree of dominance (Hillenmeyer *et al.* 2008).

In conclusion, application of a noncomplementation screen for quantitative trait alleles in yeast revealed a number of pitfalls but also insights that may help guide future studies. A primary pitfall is second-site mutations without overt phenotypes, as such mutations could be more common than is currently appreciated. However, the identification of two large-effect quantitative trait alleles indicates that quantitative noncomplementation may be well-suited to identifying alleles of large effect. Thus, quantitative noncomplementation could prove to be an excellent means of identifying alleles of large effect in any wild strain that can be mated to the yeast deletion collection without the need for extensive genotyping.

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