

# Note

## Eliminating Gene Conversion Improves High-Throughput Genetics in *Saccharomyces cerevisiae*

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### ABSTRACT

Synthetic genetic analysis was improved by eliminating leaky expression of the *HIS3* reporter and gene conversion between the *HIS3* reporter and *his3Δ1*. Leaky expression was eliminated using 3-aminotriazole and gene conversion was eliminated by using the *Schizosaccharomyces pombe his5<sup>+</sup>* gene, resulting in a 5- to 10-fold improvement in the efficiency of SGA.

**S**YNTHETIC genetic array analysis (SGA) detects genetic interactions using the collection of deletion mutants of nonessential genes in yeast (TONG *et al.* 2001, 2004). We adopted SGA for use on a small scale in laboratories that do not have access to robotics. SGA performed manually had a low success rate as judged by a number of criteria. We developed a “gold standard” set of genes that were synthetic lethal with *mad2* and showed that a typical SGA screen, using manual manipulations, identified <10% of the *bona fide* interactions. SGA efficiency improved after eliminating leaky expression of the *MFA1* promoter-driven *HIS3* reporter. Gene conversion between the *HIS3* reporter gene and the *his3Δ1* allele in strains isogenic with BY4741, BY4742, and BY4743 increased false negatives and conversion was eliminated by using the *Schizosaccharomyces pombe his5<sup>+</sup>* gene.

The success of SGA depends on being able to select haploid spores after meiosis. SGA utilizes a *MATα* query strain that contains the mutant of interest marked by a deletion with a nourseothricin resistance marker (NAT), a fusion gene with *HIS3* under the control of the *MFA1* promoter and *can1* (TONG *et al.* 2001). *MFA1* is a haploid-specific gene that is expressed only in *MATα* cells (MICHAELIS and HERSKOWITZ 1988). The query strain is mated to the collection of deletion mutants where every mutation is a deletion replaced by G418 resistance (WACH *et al.* 1994). Diploids are isolated and

sporulated, and haploids are selected on SC-his medium containing canavanine. After meiosis, populations of spores from many meioses are transferred to the haploid selection plate; the exact number depends on the sporulation efficiency of the individual cross. The haploids are then tested on medium containing NAT and G418 to determine if double mutants are inviable or compromised for growth. We constructed a query strain with *mad2::NAT*, performed SGA, and assayed for synthetic lethal interactions using manual manipulations (see supplemental material at <http://www.genetics.org/supplemental/>).

Three evaluation criteria indicated that our SGA screens were yielding limited success. The first was that three successive screens using the identical *mad2::NAT* query strain identified <10% overlap between the screens. The second indicator was that we failed to identify mutations in the full set of genes encoding proteins of multisubunit complexes. The third indicator was that we found little linkage to *MAD2* in three separate screens (TONG *et al.* 2001, 2004). To determine the overall efficiency of the *mad2* screens, we chose 170 mutants that are annotated as affecting functions relating to microtubules, cytoskeleton, kinetochore, chromosome segregation, and cell cycle (supplemental Table 1 at <http://www.genetics.org/supplemental/>). We reasoned that a subset of these mutants should compromise chromosome segregation and show a synthetic phenotype in combination with *mad2*. The mutants were mated to *mad2::NAT* and each cross was evaluated directly using random spore analysis. We identified 22 mutants that showed synthetic lethal interactions with *mad2::NAT* and served as a gold standard to judge the efficiency of a

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given SGA screen (supplemental Table 2 at <http://www.genetics.org/supplemental/>).

Two independent *mad2* screens, performed with the entire arrays of ~4700 mutants by manual pinning, identified only one and three of the known synthetic lethal interactions, respectively, and therefore detected 4 and 9% of the interactions. We picked 10 of the false negative colonies and in every case there were mixtures of colonies that were *MATa* and colonies that were sterile, suggesting they were a mixture of haploids and *MATa*/*MATα* diploids. We sporulated representative clones of the sterile colonies and the pattern of *his*<sup>+</sup> segregation after meiosis suggested that there were two *HIS3* genes (not shown). The diploids were arising because *MATα* cells were slipping through the haploid selection and then mating with the *MATa* cells that were properly selected. This happened because of leaky expression of His3 from *P<sub>MFAI</sub>-HIS3* and by *his3* reversion. The haploid query strain produced large and variable numbers of *his*<sup>+</sup> revertants (Figure 1A). The small colonies were *MATα* and were completely eliminated by adding 2 mM 3-aminotriazole to the medium (Figure 1B). The large colonies were *MATa* (29/38) or *MATα* (9/38) and were resistant to 100 mM 3-aminotriazole. The *MATa* cells arise by gene conversion between *HMRa* and *MATα*. The *MATα* colonies arose because the *his3Δ1* mutation reverted.

The BY4741, BY4742, and BY4743 strains have the *his3Δ1* allele, a 187-bp internal deletion of the *HIS3* gene. To determine if there was gene conversion of *his3Δ1*, we picked large colonies, extracted DNA, and performed PCR using primers that flank the *HIS3* gene. There is a low frequency of ectopic mitotic gene conversion between *HIS3* alleles (Figure 1C). The possibility of ectopic recombination is expected to be elevated in meiosis. It is possible that ectopic gene conversion produces *MATα HIS3* spores that can mate after germination because they would germinate in the presence of a large number of *MATa* cells that are selected in the SGA procedure (Figure 2A). To correct the problem of gene conversion, we reconstructed the strains by substituting the *S. pombe his5*<sup>+</sup> gene, which eliminated the ectopic gene conversion (data not shown).

We repeated the genomewide screen to determine if including the *S. pombe his5*<sup>+</sup> gene improved SGA, using our gold standard as an assay. Conventional SGA detected 9% of the “true” synthetic lethal interactions. The screen with the *S. pombe his5*<sup>+</sup> gene identified 82% of the possible synthetic lethal interactions. To confirm that using *P<sub>MFAI</sub>-his5*<sup>+</sup> in the query strain eliminated gene conversion we sporulated diploids derived from the *mad2::NAT* query strains crossed to the *cik1::G418* strain from the haploid collection because the double mutants are synthetically lethal (supplemental Table 2 at <http://www.genetics.org/supplemental/>). We selected haploid spores and identified rare colonies resistant to G418 and NAT, the situation that could arise by gene

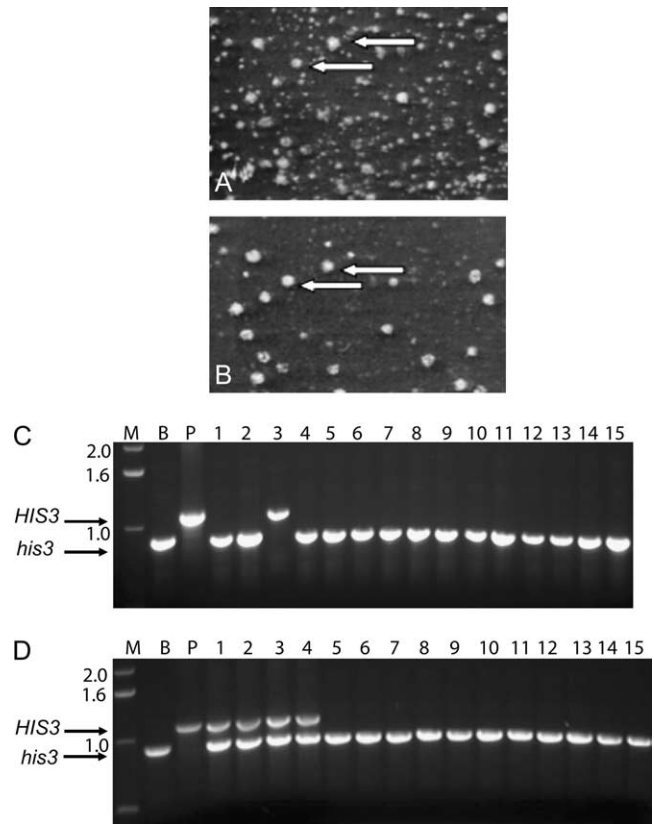


FIGURE 1.—Sources of variability in SGA. (A) *MATα* cells of strain Y2454 from an overnight culture grown in YPD were spread onto an SC-*his* plate and incubated for three days at 30°. A small region is shown. Approximately 1000 colonies of varying sizes were evident on the entire plate. (B) The same region after replica plating to a plate containing 2 mM 3-aminotriazole, which inhibits the growth of the small colonies. Arrows point to the same colonies on both plates. (C) Haploid revertants were recovered and tested for gene conversion at the *his3Δ1* locus using primers flanking the gene. Lane M is molecular weight marker, lane B is genomic DNA from BY4741 (*his3Δ1*) as template, lane P is plasmid pRS303 (*HIS3*) DNA as template, and 1–15 are DNA from the 15 revertants. All of the revertants were *MATa* except revertant 3, which was *MATα*. (D) False negative colonies were recovered from SGA after using *P<sub>MFAI</sub>-HIS3* in the query strain and tested for gene conversion at the *his3Δ1* locus, using primers flanking the gene. Lane M is molecular weight marker, lane B is genomic DNA from BY4741 (*his3Δ1*) as template, lane P is plasmid pRS303 (*HIS3*) DNA as template, and 1–15 are DNA from the 15 revertants. All of the revertants were *MATa* except 1–4, which were *MATa*/*MATα*.

conversion. We recovered rare haploids (~10<sup>-4</sup> of the spores) that were resistant to both drugs. When *P<sub>MFAI</sub>-HIS3* was used as the query gene we recovered 4 of 15 strains that were sterile, nonmatters with copies of both *his3Δ1* and *HIS3* (Figure 1D). When *P<sub>MFAI</sub>-his5*<sup>+</sup> was used as the query strain all of the colonies were *MATa* and *his3Δ1* as judged by PCR (not shown). Our success with SGA was improved by eliminating gene conversion.

The improved the SGA did not completely eliminate the false negatives. The remaining false negatives

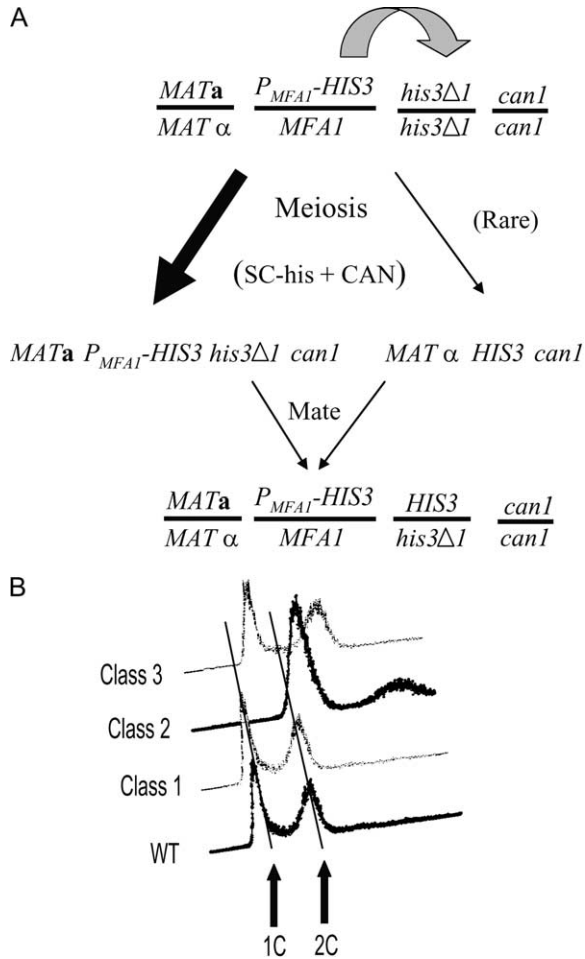


FIGURE 2.—Altered chromosome transmission events in SGA. (A) Gene conversion. A diploid in meiosis can convert either of the two *his3Δ1* alleles (shaded block arrow) prior to meiosis I. The vast majority of spores selected on SC–histidine containing canavanine (SC–his + CAN) after meiosis are *MATa P<sub>MFAI</sub>-HIS3 his3Δ1 can1* (solid block arrow, left side). Gene conversion followed by appropriate meiotic segregation can generate rare *MATα HIS3 can1* (arrow, right side). The *MATα* cells are surrounded by *MATa* cells and mate, producing diploids that escape the haploid selection and confound the SGA. (B) Flow cytometry of false negative colonies. Fifteen colonies from a *bni1* × *bni1* cross that mated with a *MATα* tester were grown to midlog, prepared for flow cytometry, and compared to BY4741. Three classes were recovered. Class 1 had an approximate haploid content of DNA, while classes 2 and 3 appeared aneuploid.

(which should be *MATa* G418- and NAT-resistant colonies) probably arose from chromosome segregation errors in meiosis. We crossed a *MATα bni1::NAT P<sub>MFAI</sub>-HIS3* query strain to *MATa bni1::G418*, sporulated the

diploid, and recovered 15 rare strains that were resistant to canavanine, G418, and NAT (false positive) and mated them with a *MATα* tester. Given that *bni1::NAT* and *bni1::G418* are alleles it should be impossible to recover haploids that contain both. We tested ploidy by two tests, flow cytometry for DNA content and pappilation to cycloheximide resistance after crossing to a *cyh2* strain. The flow cytometry profiles fell into three classes (Figure 2). One class, the majority, included cells with DNA contents indistinguishable from the haploid control (12/15). The cells pappilated to cycloheximide resistance after mating to a *cyh2* tester (data not shown) and therefore they had one copy of *CYH2* (haploid). The second class had more than double the amount of DNA (Figure 2) and did not pappilate to cycloheximide resistance (data not shown), suggesting they were diploid or aneuploid. The final class (1/15) had a DNA content that was intermediate between haploid and diploid (Figure 2) and weakly pappilated to cycloheximide resistance (data not shown). We conclude that *MATa/MATa* diploids and other aneuploids can be recovered and can confound the SGA. Regardless of the origin and number of false negatives in a screen, the important consideration is that eliminating gene conversion between *HIS3* alleles in the query strain and reducing leaky expression from the *MFAI* promoter improves the efficiency of SGA.

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