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+* gene, resulting in a 5- to 10-fold improvement in the efficiency of SGA.

SYNTHETIC 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+* 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 *MAT***a** 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, iden-
tified 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 colo-
nies that were *MAT*a and colonies that were sterile, sug-
gesting they were a mixture of haploids and *MAT*a/
*MAT*α diploids. We sporulated representative clones of
the sterile colonies and the pattern of his⁺ segregation
after meiosis suggested that there were two *HIS3* genes
(not shown). The diploids were arising because *MAT*α
cells were slipping through the haploid selection and
then mating with the *MAT*a cells that were properly se-
lected. This happened because of leaky expression of
His3 from P_{MFA1}–*HIS3* and by *his3* reversion. The
haploid query strain produced large and variable num-
bers of his⁺ revertants (Figure 1A). The small colonies
were *MAT*α and were completely eliminated by adding 2
mM 3-aminotriazole to the medium (Figure 1B). The
large colonies were *MAT*a (29/38) or *MAT*α (9/38) and
were resistant to 100 mM 3-aminotriazole. The *MAT*a cells
arise by gene conversion between *HMR*a and *MAT*α.
The *MAT*α 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 con-
version 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 *MAT*α *HIS3* spores that can mate after germi-
nation because they would germinate in the presence of
a large number of *MAT*a 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*⁺ gene, which eliminated the ectopic
gene conversion (data not shown).

We repeated the genomewide screen to determine if
including the *S. pombe his5*⁺ gene improved SGA, using
our gold standard as an assay. Conventional SGA de-
tected 9% of the "true" synthetic lethal interactions.
The screen with the *S. pombe his5*⁺ gene identified 82%
of the possible synthetic lethal interactions. To confirm
that using P_{MFA1}–*his5*⁺ 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
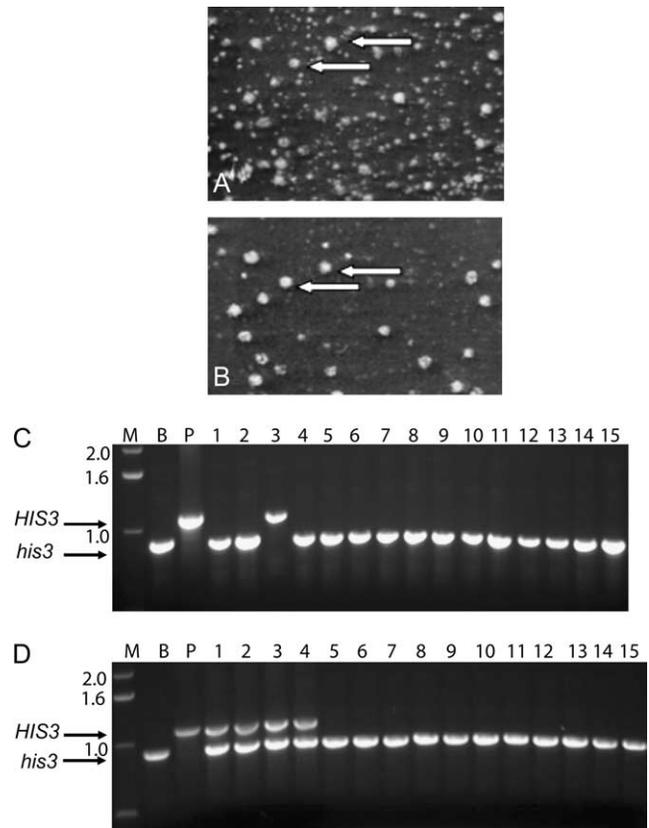G418 and NAT, the situation that could arise by gene

FIGURE 1.—Sources of variability in SGA. (A) *MAT*α 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) Hap-
loid 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 re-
vertants. All of the revertants were *MAT*a except revertant 3,
which was *MAT*α. (D) False negative colonies were recovered
from SGA after using P_{MFA1}–*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 *MAT*a except
1–4, which were *MAT*a/*MAT*α .

conversion. We recovered rare haploids (~10^{-4} of the
spores) that were resistant to both drugs. When P_{MFA1}–
HIS3 was used as the query gene we recovered 4 of 15
strains that were sterile, nonmaters with copies of both
his3Δ1 and *HIS3* (Figure 1D). When P_{MFA1}–*his5*⁺ was used
as the query strain all of the colonies were *MAT*a 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 elimi-
nate the false negatives. The remaining false negatives
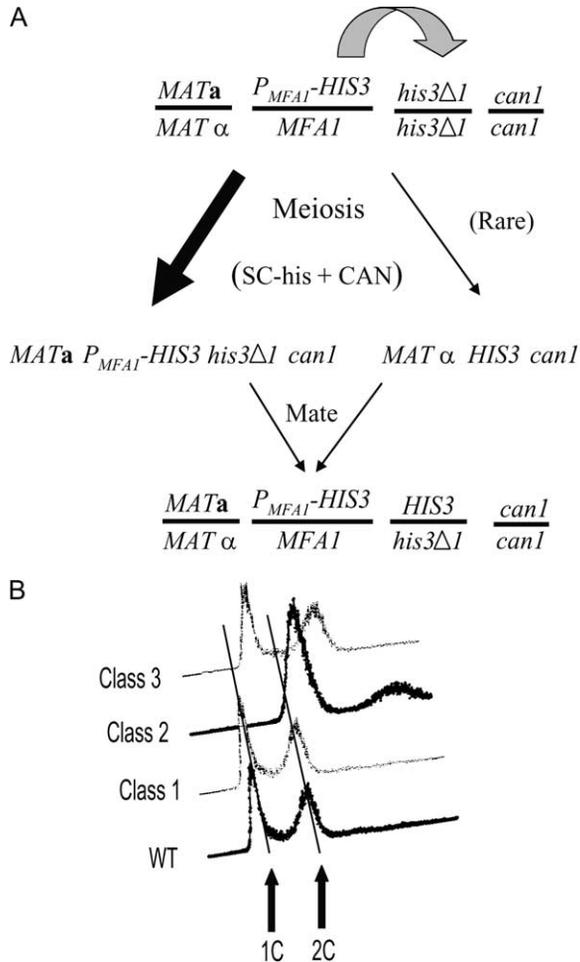
A

B

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_{MFA1}-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_{MFA1}-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 *MFA1* promoter improves the efficiency of SGA.

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