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A Robust Toolkit for Functional Profiling of the Yeast Genome

Technique

Xuewen Pan,[1,2] Daniel S. Yuan,[1,2] Dong Xiang,[5] Xiaoling Wang,[1,2] Sharon Sookhai-Mahadeo,[1,2] Joel S. Bader,[2,6] Philip Hieter,[7] Forrest Spencer,[1,3,4] and Jef D. Boeke[1,2,*]

[1]Department of Molecular Biology and Genetics
[2]The High Throughput Biology Center
[3]Department of Medicine
[4]McKusick-Nathans Institute for Genetic Medicine
The Johns Hopkins University School of Medicine
Baltimore, Maryland 21205
[5]Lineberger Comprehensive Cancer Center
University of North Carolina at Chapel Hill
Chapel Hill, North Carolina 27599
[6]Department of Biomedical Engineering
Johns Hopkins University
Baltimore, Maryland 21218
[7]Department of Biochemistry and Molecular Biology and Department of Medical Genetics
University of British Columbia
Vancouver, British Columbia V5Z 4H4
Canada

Summary

Study of mutant phenotypes is a fundamental method for understanding gene function. The construction of a near-complete collection of yeast knockouts (YKO) and the unique molecular barcodes (or TAGs) that identify each strain has enabled quantitative functional profiling of *Saccharomyces cerevisiae*. By using these TAGs and the SGA reporter, *MFA1pr-HIS3,* which facilitates conversion of heterozygous diploid YKO strains into haploid mutants, we have developed a set of highly efficient microarray-based techniques, collectively referred as dSLAM (*d*iploid-based *s*ynthetic *l*ethality *a*nalysis on *m*icroarrays), to probe genome-wide gene-chemical and gene-gene interactions. Direct comparison revealed that these techniques are more robust than existing methods in functional profiling of the yeast genome. Widespread application of these tools will elucidate a comprehensive yeast genetic network.

Introduction

The collection of yeast knockouts (YKOs) has enabled systematic genome-wide functional analyses (Giaever et al., 2002). The YKOs exist in four formats: *MAT***a**/α heterozygous diploid, *MAT***a** and *MAT*α haploids, and *MAT***a**/α homozygous diploid (Giaever et al., 2002); the latter sets contain only nonessential gene YKOs. YKO-specific molecular barcodes (or TAGs) allow quantitative functional profiling of complex populations (or pools) of YKOs using high-density oligonucleotide microarrays (Giaever et al., 2002; Winzeler et al., 1999). Such parallel analysis has vastly increased the comprehensiveness, sensitivity, accuracy, and speed of functional characterization of yeast genes.

*Correspondence: jboeke@jhmi.edu

Mutant phenotypes are most often determined by screening the haploid or homozygous diploid set of yeast YKO strains because these exhibit easily detectable phenotypes (Giaever et al., 2002; Ooi et al., 2001; Tong et al., 2001; Winzeler et al., 1999). However, these three strain sets are subject to unavoidable selection pressure and accumulation of compensatory genetic changes. As a result, strain quality can deteriorate with time, possibly delivering misleading results. For example, DNA damage response pathway mutants (*mec3*Δ, *ddc1*Δ, *rad17*Δ, *rad24*Δ, and *rad9*Δ) behaved differently in screens with homozygous diploid YKOs and haploid *MAT***a** YKOs (see Supplemental Table S1 at http://www.molecule.org/cgi/content/full/16/3/487/DC1/), giving the false impression that the corresponding genes are involved in distinct biological pathways. Haploid YKOs have been used to study gene interactions (Ooi et al., 2003; Tong et al., 2001). However, beyond their potential genetic impurity, haploid mutants in different genes behave distinctly and thus are not well suited for manipulation as a population, the basis for microarray-based TAG readouts (Ooi et al., 2003). In contrast, the mutation in each *MAT***a**/α heterozygous diploid YKO is "covered" by a wild-type copy and thus mutant phenotypes are largely to totally absent. Because of this, the genetic quality of the heterozygote YKOs exceeds that of the other YKO sets—selection for fast growing variants normally arising in haploid and homozygous diploid cultures is minimized. We show that >99% of a set of 5896 heterozygous diploid YKOs behaves more or less uniformly and thus is highly manipulable as a pool. Finally, only this set of YKOs includes essential genes, allowing simultaneous analysis of their genetic interactions.

Tong et al. (2001) described the "SGA reporter" *MFA1pr-HIS3*, which is repressed in *MAT*α haploid or *MAT***a**/α diploid cells. We integrated this *MAT***a**-specific reporter (formally referred to as *LEU2-MFA1pr-HIS3*) into the endogenous *CAN1* locus in a pool containing 5896 heterozygous diploid YKOs, resulting in cells doubly heterozygous at a gene of interest (*XXX/xxx::kanMX*) and at *CAN1* (*CAN1/can1*Δ*::LEU2-MFA1pr-HIS3*). Populations of isogenic haploid YKOs are readily generated from these "convertible" strains after sporulation and selection on appropriate media and are immediately studied for mutant phenotypes. Here we report our studies of a variety of genome-wide gene-compound and gene-gene interactions using a pool of haploid-convertible heterozygote YKOs profiled with microarrays. Direct comparison revealed that this technique, based on combining TAG array-based analysis with the superior genetic quality of the freshly converted haploid YKOs, is more robust than existing functional genomic profiling methods.

Results

A Comprehensive Gene-Chemical Interaction Analysis

The SGA reporter efficiently converts diploids to *MAT***a** haploids following sporulation and selection (Tong et

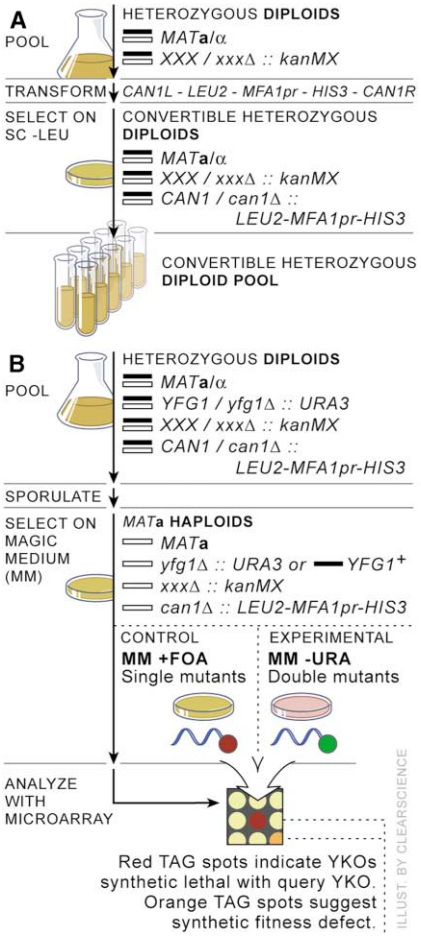

Figure 1. Analysis of Genetic Interactions with a Haploid-Convertible Heterozygous Diploid YKO Pool

(A) Construction of a haploid-convertible heterozygous diploid YKO pool. A haploid selection SGA reporter flanked by 1 kilobase sequences flanking the endogenous *CAN1* locus (*CAN1L-LEU2-MFA1pr-HIS3-CAN1R*) was transformed into a pool of 5896 heterozygous diploid YKOs to replace one copy of *CAN1*. Each of these YKOs carries an individual gene-disrupting *kanMX* module tagged with unique barcodes. This pool of YKOs was aliquoted and stored in 15% glycerol at −80°C.

(B) Diploid-based synthetic lethality analysis by microarrays (dSLAM). A query mutation (*yfg1Δ::URA3*) is introduced into the pool of haploid-convertible heterozygous diploid YKOs via high efficiency integrative transformation. Haploid single or double mutant pools are regenerated from this heterozygous diploid double mutant pool after sporulation and selection on "magic medium" (MM+5FOA or MM−Ura). Genomic DNA samples are isolated from both pools and used as the templates for PCR amplification of the TAGs labeled with fluorescent dyes (Cy5 for single mutant pool and Cy3 for the double mutant pool). Microarray analysis of these dye-labeled TAGs reveals the synthetic interaction between each of the corresponding YKOs with the query mutation. A high C/E ratio (or reddish spot on microarray) of a TAG indicates that the *yfg1Δ::URA3* mutation causes lethality or slow growth phenotype in the corresponding haploid YKO mutant.

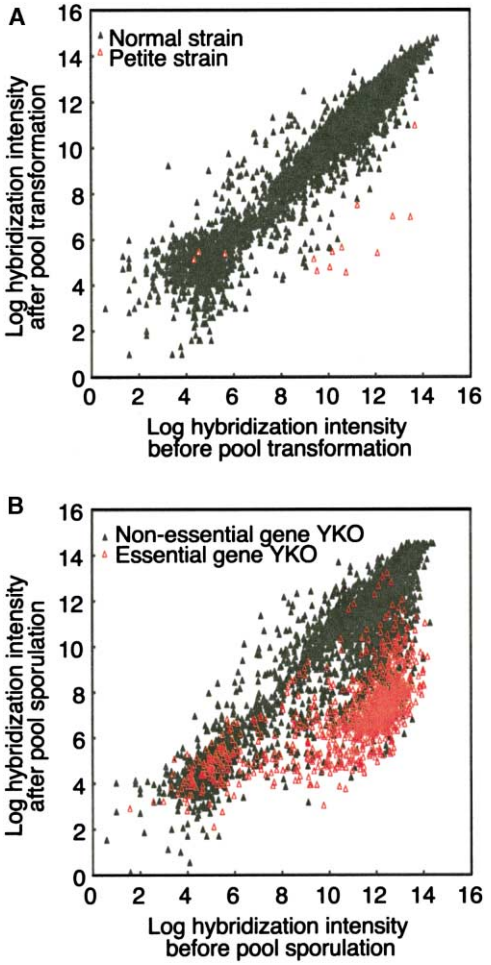

Figure 2. The Heterozygous Diploid YKOs Are Amenable to Genetic Studies as a Population

(A) The heterozygous diploid YKOs behaved uniformly during an integrative transformation. The *CAN1L-LEU2-MFA1pr-HIS3-CAN1R* cassette featuring the SGA reporter was transformed into a pool of 5896 heterozygous diploid YKOs. Genomic DNA was isolated from the starting pool and the transformed pool. The abundance of each YKO in both pools was compared by microarray analysis of the TAGs. Normalized Log2 hybridization signal intensities of the UPTAGs were plotted (DOWNTAGs were similar). Known petite strains, open red triangles; other YKOs, filled black triangles.

(B) Freshly converted haploid essential gene YKOs are underrepresented after sporulation. A pool of 5896 heterozygous diploid YKOs harboring the SGA reporter were sporulated and grown in the haploid selection magic medium (MM). The abundance of each mutation in the heterozygous diploid and freshly generated haploid pools was compared by microarray analysis of the representing TAGs. Normalized Log2 UPTAG hybridization intensities were plotted (DOWNTAGs were similar). YKOs of "essential" genes, open red triangles; YKOs of nonessential genes, filled black triangles. The large cluster in the lower left corner presumably represents mutated TAGs (Eason et al., 2004).

al., 2001). We further determined that ~99.9% of the freshly converted cells behave as true haploid *MAT***a** cells (Supplemental Figure S1). We thus constructed a pool of 5896 strains from the existing heterozygote YKO collection and integrated the SGA reporter into it en masse (Figure 1A). This YKO pool behaved quite uniformly during genetic manipulation because <1% (54 of 5896) of the YKOs, including 13 preexisting petite strains, disappeared from the pool following transformation, and >93% of the mutants exhibited <20% variations of hybridization intensity before and after transformation (Figure 2A). The convertible heterozygote pool is efficiently converted to haploid *MAT***a** YKOs for phe-

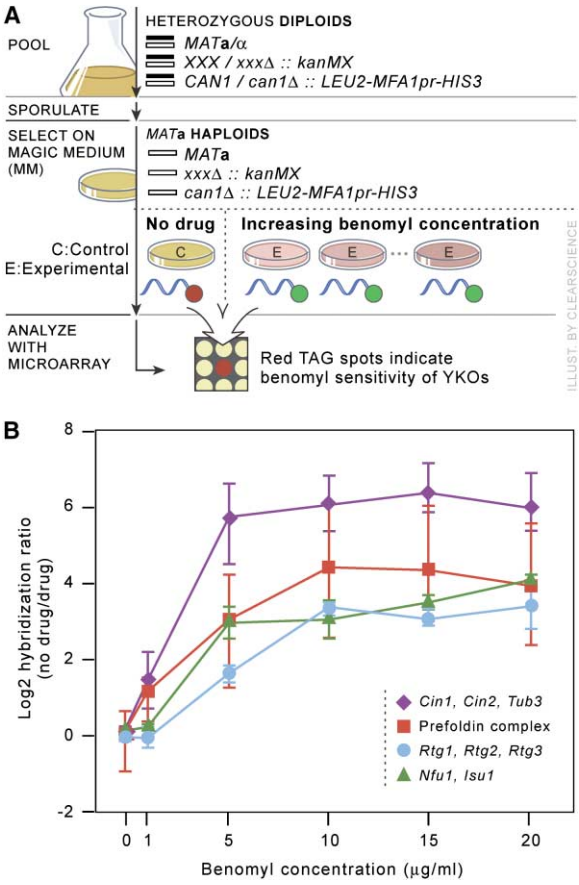

Figure 3. Systematic Genetic Screens for Benomyl-Sensitive YKOs

(A) A procedure of genetic screens for benomyl-sensitive YKOs with the haploid-convertible pool. An aliquot of the heterozgyous diploid YKOs bearing the SGA reporter was sporulated. Haploid pools were freshly regenerated on basic haploid selection medium (MM) with (experimental) or without (control) increasing concentrations of benomyl and analyzed by microarrays. High C/E ratios revealed benomyl-sensitive YKOs.

(B) Benomyl sensitivity of a subset of YKOs reflected by the log2 hybridization C/E ratios (no drug/drug). The sensitivity of the indicated YKOs to increasing concentrations (0 to 20 μg/ml) of benomyl was plotted. For simplicity, TAG hybridization C/E ratios of YKOs at indicated benomyl concentrations were averaged within a functional group: microtubule biogenesis (*CIN1, CIN2*, and *TUB3*); Gimc prefoldin complex (*GIM3, GIM4*, *GIM5*, *PAC10*, *PFD1*, and *YKE2*); *NFU1* and *ISU1*; *RTG1, RTG2*, and *RTG3*. Data for this plot were derived from Supplemental Table S2.

notypic analysis as demonstrated by the significant underrepresentation of ~96.6% (998) of the 1032 assayable essential gene mutations in a freshly generated haploid pool (Figure 2B).

A genome-wide screen with *MATa* haploid YKOs recently identified 78 nonessential genes required for benomyl resistance in yeast (Parsons et al., 2004). To evaluate the utility of our haploid-convertible heterozygote pool for high-throughput genetic screens, we exposed a freshly converted haploid YKO pool to various concentrations of benomyl (experimental pool). Representation of each YKO was compared to a control pool generated in the absence of benomyl using TAG array analysis (Figure 3A). A high control/experimental hybridization signal ratio (referred to as the C/E ratio) indicates that the corresponding YKO is sensitive to benomyl. With a cutoff C/E ratio of ≥2.0, we identified ~450 candidate benomyl-sensitive YKOs (Supplemental Table S2). Thirty-seven of seventy-eight previously identified mutants, most of which exhibited moderate or weak benomyl sensitivity in the previous screen, were not reidentified here, possibly due to different media used in the two screens.

As expected, our screen revealed that mutants of genes directly required for microtubule biogenesis exhibited hypersensitivity to low concentrations of benomyl. These include *cin1*Δ, *cin2*Δ, *cin4*Δ, *gim3*Δ, *gim4*Δ, *gim5*Δ, *pac10*Δ, *pfd1*Δ, *tub3*Δ, and *yke2*Δ (Figure 3; Supplemental Table S2; and data not shown) YKOs. The *pac2*Δ mutant was absent from the pool used (Supplemental Table S3) but was subsequently shown to be hypersensitive to benomyl. Strikingly, we found concordant phenotypes of different mutants affecting the same biological activity; such consistency was not observed in the study of the haploid YKOs. For example, Gim3, Gim4, Gim5, Pac10, Pfd1, and Yke2 constitute the GimC prefoldin complex (Siegers et al., 1999), and we found similar benomyl hypersensitivity among the corresponding haploid mutants freshly derived from the isogenic heterozygous diploid YKOs. In contrast, the existing *MATa* haploid YKOs of these genes exhibited different levels of sensitivity to benomyl: most were hypersensitive, but the *pfd1*Δ mutant showed moderate sensitivity, and the *gim4*Δ mutant showed weak sensitivity to benomyl (Parsons et al., 2004). Cin1, Cin2, Cin4, and Pac2 stabilize the tubulin heterodimers (Fleming et al., 2000), and the *cin1*Δ, *cin2*Δ, *cin4*Δ, and *pac2*Δ mutants were similarly hypersensitive to benomyl in our study. Again, different levels of benomyl sensitivity were observed among these four mutants by Parsons et al. (2004). In addition, we reidentified all known spindle and mitotic checkpoint mutants (*mad1*Δ, *mad2*Δ, *mad3*Δ, *bub1*Δ, *bub2*Δ, *bub3*Δ, and *bfa1*Δ) sensitive to benomyl (Supplemental Table S2), whereas the previous haploid-based screen failed to identify *mad3*Δ, *bub2*Δ, and *bfa1*Δ (Parsons et al., 2004). We have also identified new *groups of genes* required for benomyl resistance in wild-type yeast. These include *ISU1* and *NFU1,* involved in mitochondrial iron homeostasis, *RTG1*, *RTG2*, and *RTG3* of the retrograde response pathway, and most negative regulators of the cAMP signaling pathway (*GPB1*, *GPB2*, *IRA1*, *IRA2*, and *PDE2*) (Figure 3; Supplemental Table S2).

These results suggest that some existing *MATa* haploid YKOs harbor cryptic mutations that compensate for benomyl-sensitive phenotypes, or perhaps these strains have been cross-contaminated by benomyl-insensitive strains. Alternatively, intrinsic variations in the colony-based high-throughput screen method itself could be responsible. In contrast, haploid mutants derived from the heterozygous diploid YKOs behaved as expected and are less likely to have accumulated compensatory mutations. The TAG-array analysis is fast and immune to strain cross-contamination, which can in principle hamper individual colony assays. Thus, our TAG-array analysis of the haploid-convertible heterozygous diploid YKOs is well suited for studying genome-wide YKOs' response to chemicals and other physiological stresses.

dSLAM Analysis of Gene-Gene Interactions

Systematic analysis of gene-gene interaction with the *s*ynthetic *g*enetic *a*rray (SGA) has dramatically increased our understanding of genetic complexity and gene functions in yeast (Tong et al., 2001, 2004). However, SGA is limited by genetic instability of existing haploid mutants, possible cross-contaminations between strains, mating defects of some YKOs, and other practical issues like difficulty in controlling inoculum sizes between control and experimental samples. As shown below, SGA creates high false negative rates. Moreover, SGA is relatively slow and laborious, and may be more expensive compared to array-based techniques (Ooi et al., 2003). In an attempt to speed up the analysis of genetic interaction across the yeast genome, we previously developed a haploid-based *s*ynthetic *l*ethality *a*nalyzed by *m*icroarray (SLAM) method for studying gene-gene interactions genome-wide (Ooi et al., 2003). This method, based on TAG-array analysis, efficiently identified colethal mutant pairs (Ooi et al., 2003). However, haploid SLAM is hampered by phenotypic heterogeneity of haploids, low integration accuracy (~85%), and inefficient integration of the query construct (Ooi et al., 2003). As a result, haploid SLAM is relatively insensitive, as it failed to identify a number of known interactions. Moreover, haploid SLAM does not work well with complete deletions of genes >500 bp, further limiting its usefulness (Ooi et al., 2003).

Heterozygotes are highly amenable to population-based manipulation (Figure 2), and moreover, integrative transformation accuracy of 97.5%–99.5% was observed at four loci (Supplemental Figure S2). Approximately 10-fold higher transformation efficiencies were obtained in the heterozygote pool with 12 different YKO cassettes (Supplemental Figure S3). These transformation yields were achieved using a *URA3* version of the preexisting YKO cassettes (Supplemental Figure S3), facilitating re-engineering of query constructs (haploid SLAM requires special cassettes deleting <500 bp) (Ooi et al., 2003). Such practical factors become highly significant when contemplating a comprehensive 5000 × 5000 genetic interaction map.

A heterozygote *d*iploid-based *s*ynthetic *l*ethality *a*nalysis with *m*icroarray (dSLAM) protocol was developed and used to query genome-wide gene-gene interactions. A *yfg1*Δ*::URA3* query construct containing 1.5 kb *YFG1* (*YFG1* stands for *y*our *f*avorite *g*ene 1) flanking sequences is transformed into the heterozygote pool. Pools of *yfg1*Δ*::URA3 xxx*Δ*::kanMX* double mutant and *YFG1*$^+$ *xxx*Δ*::kanMX* single mutant haploids are generated by sporulation and selection of the transformed pool; relative YKO abundance in the two pools was deduced from TAG arrays. High C/E ratios between single/double YKO pools indicate a synthetic fitness defect with *yfg1*Δ. To compare dSLAM to SGA and haploid SLAM, we compared our dSLAM analyses, using *cin8*Δ, *bim1*Δ, and *sgs1*Δ queries, with previous studies (Ooi et al., 2003; Tong et al., 2004).

We performed three independent *cin8*Δ synthetic lethality screen experiments with our dSLAM protocol. Unsurprisingly, uracil auxotrophs had high C/E ratios in such screens because they cannot grow on the haploid double mutant selection medium (Supplemental Table S4). In addition, mutants immediately flanking *CIN8* (*npr2*Δ and *prb1*Δ) had predicted high C/E ratios because their TAGs overlap the *cin8*Δ*::URA3* query cassette and are underrepresented in the double mutant pool (Supplemental Table S4). In contrast, the *cin8*Δ mutant itself has a low C/E ratio (Supplemental Table S4) because the query cassette contains the *cin8* TAGs (Supplemental Figure S3) and is vastly overrepresented in the double mutant pool. These three mutant types (uracil auxotrophic mutants, query mutant, and adjacent mutants) serve as internal controls for each dSLAM screen.

59 *cin8*Δ synthetic interactions with nonessential genes were previously identified by SGA and more conventional genetic screens (Geiser et al., 1997; Tong et al., 2004). With a C/E ratio cutoff of ≥2.0 in all three experiments, we identified 193 potential *cin8*Δ synthetic interactions, and 116 were individually confirmed as correct by tetrad analysis on YPD and random spore analysis on synthetic medium (Supplemental Table S5). These include 43 previously identified and 73 new ones (Figure 4A; Supplemental Table S5). Sixteen *cin8*Δ interactions identified by SGA and conventional methods were missed when the ≥2.0 cutoff C/E ratio was applied (Figure 4A; Supplemental Table S5). Thus, a total of 132 *cin8*Δ synthetic interactions have now been identified. Among the 16 interactions missed here, *pac2*Δ was absent from the pool (Supplemental Table S3); *rts1*Δ and *mrpl36*Δ had low hybridization intensities and no data were available (Supplemental Table S5). Eight others had signal hybridization C/E ratios ≥1.5 in two or more screens, and we subsequently confirmed a synthetic fitness defect with *cin8*Δ by tetrad dissection on YPD (Supplemental Table S5). These results indicate that, by taking a ≥2.0 cutoff C/E ratio in all three experiments, some other potential *cin8*Δ synthetic interactors might have been missed. As discussed below, there is always a tradeoff between false positive rate and false negative rate in a dSLAM screen in this "gray zone."

For each of the 193 YKOs with a C/E ratio ≥2.0 in all three *cin8*Δ synthetic lethality screens, a mean C/E ratio was obtained for the three experiments. False positive and false negative rates corresponding to different cutoff mean C/E ratios were then determined. For mutants with a mean C/E ratio ≥6.0, 40 of 41 potential *cin8*Δ interactions were confirmed as correct, producing 2.3% false positives (Figure 4B; Supplemental Table S5). However, such a high stringency produces 69.7% false negatives (92 of 132 interactions missed). By reducing cutoff stringency, we uncovered more true interactions, but false positive rates rose (Figure 4B). With a cutoff C/E ratio ≥2.0, we had a false negative rate of 12.1% and a false positive rate of 39.9% (Figure 4B). SGA screens previously identified 59 *cin8*Δ synthetic lethal or fitness interactions (Tong et al., 2004), with a false negative rate of 55.3% (73 of 132 were missed). Thus the *cin8*Δ dSLAM screens had a lower false negative rate than the SGA screens (Figure 4C). More importantly, our *cin8*Δ dSLAM screens achieved better data reproducibility than the SGA screens. With a ≥2.0 cutoff C/E ratio, any single run of dSLAM uncovered at least 87.9% of the *cin8*Δ synthetic interactions (Figure 4D). In contrast, among the 59 *cin8*Δ synthetic interactions identified by SGA, only 26 were scored as positive in all three experimental runs (Tong et al., 2004). Thus only ~20% of the 132

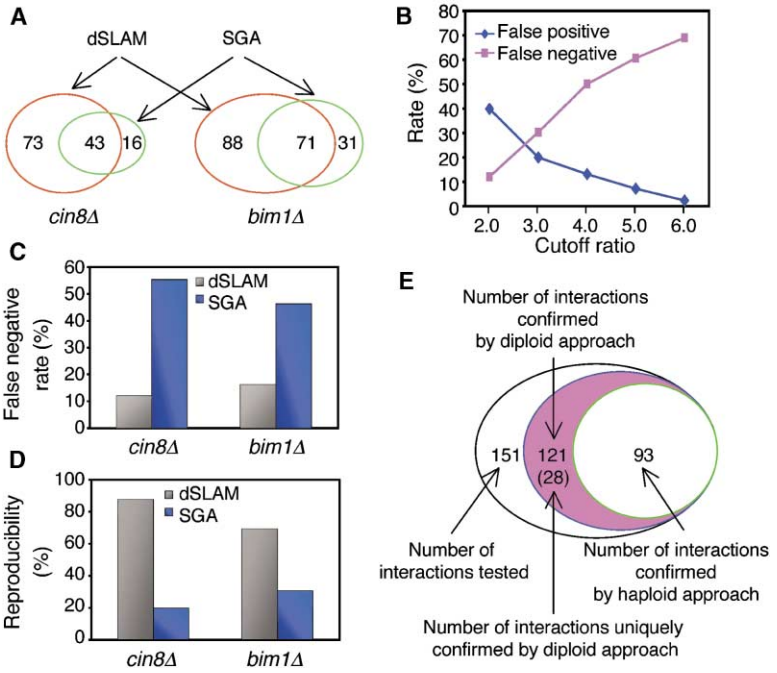

Figure 4. A Direct Comparison between dSLAM and SGA

(A) The number of *cin8*Δ and *bim1*Δ synthetic interactions identified by dSLAM and SGA.

(B) False positive and false negative rates for the *cin8*Δ dSLAM screen with regard to hybridization cutoff C/E ratios.

(C) Comparison of the false negative rates between dSLAM and SGA with *cin8*Δ and *bim1*Δ screens as examples.

(D) Comparison of data reproducibility between dSLAM and SGA with *cin8*Δ and *bim1*Δ screens as examples. Data for the plots in this figure were adopted from Supplemental Tables S5 and S6 and Tong et al. (2004).

(E) Effects of the YKO libraries on individual confirmation test of dSLAM results. The top 151 candidate *bim1*Δ synthetic interactions initially identified by dSLAM were individually tested by transforming a *bim1*Δ*::URA3* cassette into the *MAT***a**/α heterozygous diploid YKOs or by crossing a *MAT*α *bim1*Δ*::URA3* query strain to the preexisting haploid *MAT***a** YKOs. The resultant heterozygous diploid double mutants were studied for synthetic interactions between *bim1*Δ and the candidate YKOs by tetrad dissection and random spore analysis. Of the 151 interactions tested (black oval), 121 were confirmed by the diploid approach (blue oval), whereas 93 were confirmed by the haploid approach (green oval). Thus, 28 *bim1*Δ interactions (pink-shaded) could be confirmed only by using the *MAT***a**/α heterozygous diploid YKOs.

*cin8*Δ synthetic interactions were definitely uncovered by a single SGA screen (Figure 4D).

This difference in false negative rates and data reproducibility between dSLAM and SGA was also observed in *bim1*Δ synthetic lethality screens. Two independent dSLAM experiments were performed. By taking a ≥1.5 cutoff C/E ratio in both experiments, we uncovered 159 *bim1*Δ synthetic interactions that were individually confirmed by tetrad dissection on YPD or random spore analysis on synthetic medium (Supplemental Table S6). In comparison, SGA screens identified 102 *bim1*Δ interactions (Tong et al., 2004). The dSLAM and SGA *bim1*Δ screens share 71 common interactions and bring the total number of *bim1*Δ interactions to 190 (Figure 4A). dSLAM has a false negative rate of 16.3% (31 of 190), whereas SGA has a false negative rate of 46.3% (88 of 190) (Figure 4C). We believe that the relatively high false negative rates of the SGA *bim1*Δ screen are partly due to genetic impurity of the haploid *MAT***a** YKO library used. In the early stages of the development of the dSLAM method, we confirmed 93 of the top 151 *bim1*Δ interactions on the hit list by using an individual *MAT***a** YKO library strain. In addition to these 93 interactions, 28 more were found to be true *bim1*Δ interactions when *MAT***a**/α heterozygote YKOs were used in individual tests (Figure 4E). Interestingly, these 28 interactions were also missed in the SGA screen, likely due to problems associated with the *MAT***a** YKO library strains. There was also a large difference in data reproducibility between the dSLAM and SGA *bim1*Δ screens. 68.4% (130 of 190) of *bim1*Δ interactions had a signal hybridization C/E ratio ≥1.5 in two runs of the dSLAM screen (Figure 4D; Supplemental Table S6). Approximately 30.5% (58 of 190) were scored as positive in at least two of three experiments (Tong et al., 2004) (Figure 4D). These data indicate lower false negative rates and better data reproducibility for dSLAM than SGA.

dSLAM failed to identify 31 previously identified *bim1*Δ interactions (Supplemental Table S6). We randomly selected 15 of those 31 mutations for testing and did not observe detectable *bim1*Δ synthetic interaction with *nbp2*Δ or *inp52*Δ with either random spore analysis on the haploid selection magic medium or by tetrad analysis on YPD (Supplemental Table S6). We did see various degrees of synthetic fitness defects between *bim1*Δ and the other 13 mutations tested by tetrad analysis on YPD. Surprisingly, these defects were hardly detectable in random spore analysis on haploid selection medium (Supplemental Table S6), suggesting that some synthetic interactions are YPD specific and not obvious on the synthetic haploid selection medium.

We have also performed three independent *sgs1*Δ synthetic lethality screens with dSLAM and identified 60 synthetic lethal or fitness *sgs1*Δ interactions (Supplemental Table S7). All these interactions were individually confirmed by random spore analysis on magic medium. In comparison, the SGA screen identified 25 and haploid SLAM identified 13 *sgs1*Δ synthetic interactions (Ooi et al., 2003; Tong et al., 2004). Thus our dSLAM screens have identified a more complete set of *sgs1*Δ synthetic interactions than either SGA or haploid SLAM.

It is hard to compare false positive rates between our dSLAM screens and the SGA screens. A 25%–50% false positive rate has been reported for any given SGA screen (Tong et al., 2004); however, this number is probably biased, because for interactions that scored positive in one of three SGA screens, only mutants known to be involved in similar biological processes were selected for followup testing (Tong et al., 2004). Thus, with quantitatively ranked hit lists, low false negative rates, and

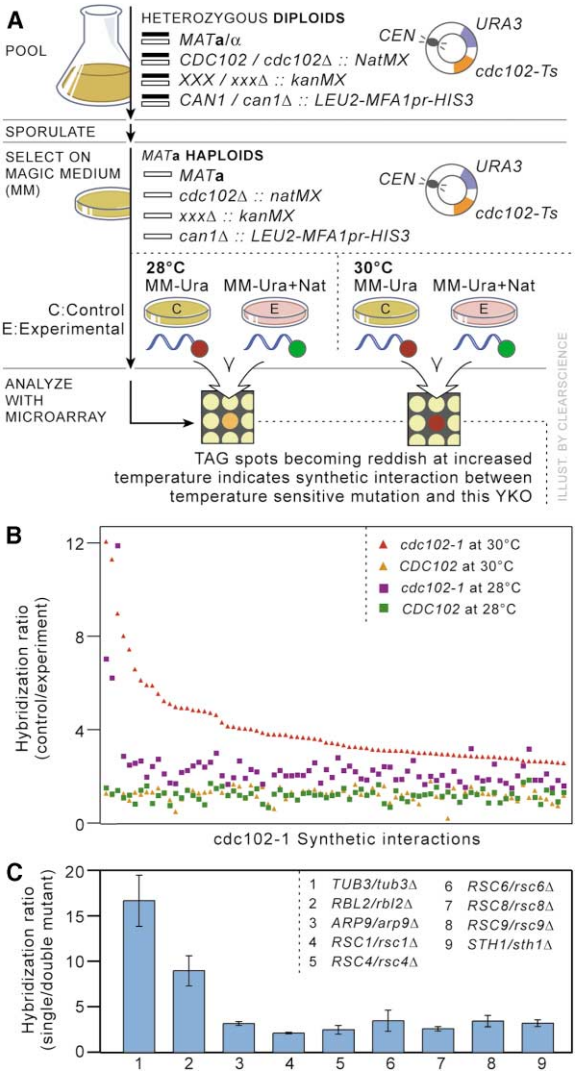

Figure 5. Synthetic Interaction Analysis Concerning Essential Genes

(A) dSLAM using a thermal sensitive (Ts) query allele of the essential gene *CDC102* (*cdc102-1*). One copy of the *CDC102* gene in the heterozygous diploid pool was replaced with a *natMX* cassette that confers resistance to CloNat (Nat). The resultant pool of diploid double YKOs was transformed with a centromere (CEN)-based plasmid that carries the *cdc102-1* allele and was marked with *URA3*. Haploids were freshly generated from this pool after sporulation and selection on different media at semipermissive temperatures as indicated and analyzed by dSLAM.

(B) Synthetic interaction identified with the *cdc102-1* Ts allele as the query. Sporulated double YKO pools carrying the wild-type *CDC102* gene and *cdc102-1* on a centromere-based plasmid were independently germinated on a haploid selection medium with (MM−Ura+Nat; double YKO pool as experiment) or without (MM−Ura; single and double YKO pool as control) 50 μg/ml CloNat at 28°C or 30°C. The growth of each YKO in the control and experimental pool was compared by microarray analysis. The C/E ratio that represents the relative strength of interaction between each of 80 YKO mutations and both *CDC102* alleles was plotted.

(C) Synthetic haplo-insufficiency screen with *tub1Δ::URA3* as the query mutation. One copy of the essential *TUB1* gene was replaced with a *URA3* dominant selectable marker in the heterozygous diploid YKO pool by homologous recombination to create an experimental pool (TUB1/tub1Δ*::URA3 XXX/xxx*Δ*::kanMX*). A *ura3*Δ*::URA3* cassette was transformed back into the endogenous *URA3* locus to create a control pool (*URA3/ura3*Δ*0 XXX/xxx*Δ*::kanMX*). The growth of each YKO in the control and experimental pools was compared by microarray analysis. The Log2 hybridization C/E ratios of a set of confirmed *tub1*Δ synthetic haplo-insufficiency interactions were plotted.

excellent data reproducibility, we believe dSLAM is the most robust technique for analyzing genome-wide synthetic lethality in yeast.

Suppression of Essential Gene Mutations

The heterozygous diploid YKO pool contains essential gene YKOs and offers an opportunity to examine suppression of lethality in parallel with a synthetic lethality screen. Instead of seeking for synthetic fitness mutations underrepresented in the double YKO pool (a *high* C/E ratio), growth defects of certain mutations suppressed by a second mutation could be sought (revealed by a *low* C/E ratio) (Figure 6A). To test this idea, we performed a synthetic lethality analysis with *sml1*Δ*::URA3* as the query. *SML1* encodes for a ribonucleotide reductase inhibitor that represses dNTP synthesis (Zhao et al., 1998). Mutation of *SML1* restores viability to *mec1*Δ, *lcd1*Δ, and *rad53*Δ YKOs defective in DNA damage response (Paciotti et al., 2000; Rouse and Jackson, 2000; Zhao et al., 1998). We did not find any *sml1*Δ synthetic lethal interactions in the screen. However, the relative abundances of the *mec1*Δ, *lcd1*Δ, and *rad53*Δ YKOs were dramatically increased in the double YKO pool as compared to the single YKO pool (Figure 6B), indicating the usefulness of this approach to study the suppression of lethality caused by essential gene knockout. In fact, their C/E ratios were the lowest ones on the list. When other potential mutants were individually tested, we identified a fourth YKO, *erd2*Δ, which is not lethal but has low efficiency of plating; this plating efficiency defect is partially suppressed by the *sml1*Δ YKO (data not shown).

Synthetic Interaction Screen with a Conditional Allele of an Essential Gene

Essential genes function in critical biological processes; their relationship to other genes is very important for a complete understanding of the wiring diagram of life. As outlined above, it is possible to find bypass suppressors of knockout mutations in essential genes. However, this represents a very limited subset of essential gene interactions. Conditional alleles are much more powerful far-reaching probes of essential gene function. Here we describe a screen of synthetic lethality interactions between a conditional allele of an essential gene and the genome-wide collection of YKO mutations. *CDC102* is an essential gene required for DNA replication in yeast (Kanemaki et al., 2003; Takayama et al., 2003). To study the synthetic interactions between a *cdc102* mutation and the set of YKOs, a *cdc102*Δ*::natMX* cassette conferring Nourseothricin resistance was en masse transformed to replace one copy of the wild-type *CDC102* gene in the convertible heterozygous diploid pool. A pool of heterozygote YKOs was generated, harvested, and retransformed with a wild-type or thermosensitive (Ts) allele of the *CDC102* gene (*cdc102-1*) on a *URA3 CEN* plasmid. Two sequential transformations were em-

ployed in this protocol. However, no significant change in the composition of the population of YKOs was observed after the second transformation because plasmid transformation was very efficient and $>5 \times 10^6$ Ura$^+$ transformants were obtained for each transformation. Control (single and double YKO; *CDC102 xxx*Δ*::kanMX* and *cdc102*Δ*::natMX xxx*Δ*::kanMX*) and experimental (double YKO; *cdc102*Δ*::natMX xxx*Δ*::kanMX*) haploid YKO pools were generated for microarray analysis after sporulation and selection at semipermissive temperatures for *cdc102-1* (Figure 5A).

We identified ~80 potential *cdc102-1* synthetic interactions, and most were more prominent at 30°C than at 28°C, consistent with reduced *cdc102-1* activity at higher temperatures (Figure 5B; Supplemental Table S8). Some of the interactions identified genes involved in cell cycle control: *CKB1* and *CKB2* are required for G1-to-S phase transition in the cell cycle, and *LTE1* is required for mitotic exit (Bardin et al., 2000; Russo et al., 2000; Shou et al., 1999). Interestingly, *cdc102-1* also exhibited synthetic interactions with both *ptc1*Δ and *nbp2*Δ, and Ptc1 and Nbp2 physically interact (Ho et al., 2002; Ito et al., 2000; Uetz et al., 2000). These results indicate that dSLAM is easily modified to study genetic interactions between an allele of an essential gene and other gene YKOs.

Dosage Synthetic Interaction or Suppression

Dosage synthetic interaction is a phenomenon in which overexpression of a gene enhances the phenotypes of a mutation in another gene, whereas dosage suppression means phenotypic compensation for that mutation. Here we describe a combined systematic dosage synthetic interaction and suppression screen with overexpression of the *LCD1* gene using the convertible heterozygote pool.

Lcd1 binds to Mec1, an essential phosphatidylinositol kinase-like protein kinase that plays a central role in DNA damage response and DNA replication, and is required for bringing Mec1 to sites of damaged DNA and its substrates in response to DNA damage (Paciotti et al., 2000; Rouse and Jackson, 2000, 2002). Cells overexpressing *LCD1* fail to response to DNA damage and are sensitive to MMS and UV radiation (Paciotti et al., 2000). *LCD1* overexpression caused a growth defect in YKOs of *ASF1*, *MMS1*, *RTT101*, *RTT109*, *RAD27*, *CTF4*, *CSM3*, *TOF1*, and *YDL162C*, which reduces expression of the adjacent DNA ligase gene *CDC9* (Figure 6C). An additional *LCD1* dosage synthetic interaction includes *lyp1*Δ. These results were individually confirmed; thus, the haploid-convertible heterozygote YKOs can be used to study genome-wide dosage synthetic interactions.

In this *LCD1* dosage interaction screen, hybridization signal of the *lcd1*Δ YKO TAGs increased when *LCD1* was overexpressed (Figure 6C). Because the *LCD1* overexpression plasmid itself lacked the *lcd1*Δ TAGs, this reveals the possibility of finding essential gene YKOs whose viability is restored when a second gene is overexpressed. We did not find such an essential gene mutation in this *LCD1* overexpression dosage interaction screen, possibly because such a mutation does not exist.

Synthetic Haplo-Insufficiency Screen

A less common genetic interaction in yeast is synthetic haplo-insufficiency (also called unlinked noncomplementation [Stearns and Botstein, 1988]), in which two heterozygous mutations individually lack phenotypes but when combined produce phenotypes. This genetic interaction often occurs between genes with important structural functions and can be exploited to study genetic interactions, including those between two essential genes. Here we report a systematic synthetic haplo-insufficiency (SHI) screen with *tub1*Δ*::URA3* as a query mutation.

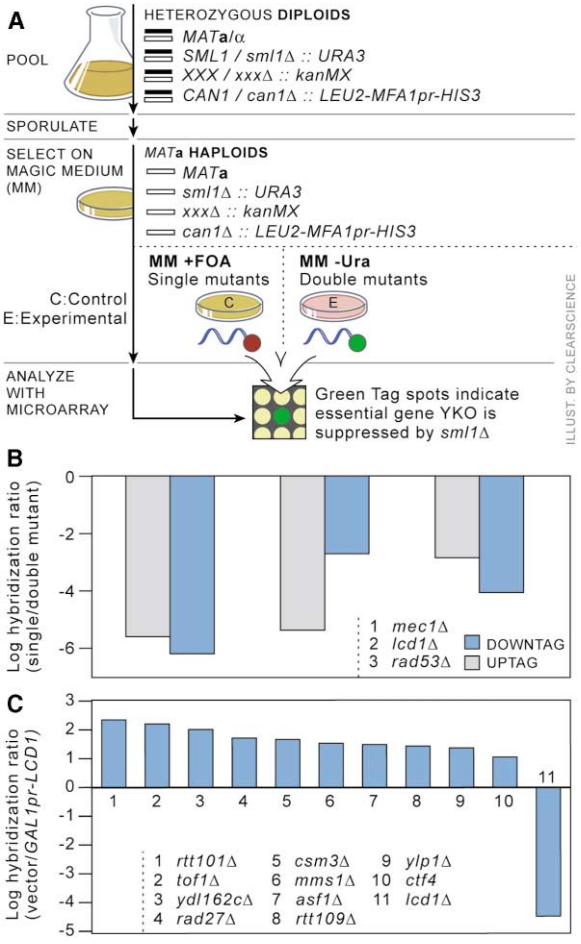

Figure 6. Other Systematic Gene-Gene Interaction Screens

(A) Screen for essential gene YKOs suppressed by the query mutation in a dSLAM experiment. A synthetic lethality screen with *sml1*Δ*::URA3* as the query mutation was performed. A *low* C/E ratio of a TAG (or green spot on microarray chip) indicates that a growth defect caused by the YKO is suppressed by *sml1*Δ*::URA3*.

(B) Suppression of *mec1*Δ, *lcd1*Δ, and *rad53*Δ mutations by *sml1*Δ. The Log2 C/E ratios of the TAGs representing *mec1*Δ, *lcd1*Δ, and *rad53*Δ were plotted.

(C) Dosage synthetic lethality and suppression screen with *LCD1* overexpression. A pool of heterozygous diploid YKOs with the SGA reporter was independently transformed with a control vector (YCplac33) and a *LCD1* overexpression plasmid (pXP465; *GAL1pr-LCD1*). The two resultant pools were sporulated and spread on a haploid selection medium (MM) with galactose as the sole carbon source to induce *LCD1* overexpression. The growth of each regenerated haploid *MAT*a haploid YKO in these two pools was compared by microarray hybridization of the representing TAGs. A high C/E ratio of vector/*GAL1pr-LCD1* indicates a growth defect of the YKO caused by *LCD1* overexpression. A low C/E ratio reflects suppression of the growth defect of that YKO by *LCD1* overexpression. Log2C/E ratios of a set of individually confirmed interactions were plotted.

TUB1 is an essential gene encoding α-tubulin, which together with β-tubulin (encoded by *TUB2*) forms the major constituent of microtubule (Water and Kleinsmith, 1976). A SHI interaction has been reported between mutations in *TUB1* and *TUB3*, a nonessential α-tubulin gene functionally identical to *TUB1* (Stearns and Botstein, 1988). In addition to the *tub3*Δ mutation, we found a new SHI interaction between *tub1*Δ and *rbl2*Δ (Figure 5C), a mutation affecting β-tubulin folding. Free β-tubulin molecules not in heterodimers with α-tubulin disrupt microtubule assembly and are toxic to yeast cells. Rbl2 binds to free β-tubulin and helps converting it into an aggregated nontoxic form (Abruzzi et al., 2002). Free β-tubulin probably accumulates in the *TUB1/tub1*Δ *TUB3/tub1*Δ and *TUB1/tub1*Δ *RBL2/rbl2*Δ heterozygous double YKOs, inhibiting their growth. Additional *tub1*Δ-interacting mutations included *arp9*Δ, *rsc1*Δ, *rsc4*Δ, *rsc6*Δ, *rsc8*Δ, *rsc9*Δ, and *sth1*Δ, all known components of the essential chromatin-remodeling activity RSC (Cairns et al., 1996; Sanders et al., 2002) (Figure 5C). Several of these mutations define essential genes. Therefore, the SHI screen is a simple method allowing detection of essential genetic interactions using only YKO mutations. Practically, the synthetic haplo-insufficiency screen could be an economical approach to identify essential gene interactions. The heterozygote diploid double YKO pool generated during a synthetic lethality screen can be directly analyzed without sporulation for synthetic haplo-insufficiency.

Discussion

Here we described a pool of a nearly complete set of haploid-convertible heterozygous diploid yeast YKOs and have developed a versatile set of protocols to systematically study a wide variety of genetic interactions with this YKO pool. By combining the strength of both TAG-array analysis and the high genetic fidelity and molecular manipulability of the heterozygous diploid yeast YKOs, our technique has proven more robust than existing methods in functional profiling the yeast genome. In the benomyl-sensitivity screen, we have found that mutants compromising the same biological activity (e.g., the prefoldin complex) show similar benomyl sensitivity profiles. This phenotypic consistency is extremely important for functional grouping of genes during high-throughput analyses of the yeast genome, and this would be difficult by studying the existing haploid YKOs. In addition, we have identified many previously unknown benomyl-sensitive mutants. In comparison to existing methods, our dSLAM screens achieved high data reproducibility, low false negative rates, and, potentially, lower false positive rates (Figure 4). With subtle modifications of dSLAM, we have studied various types of genome-wide genetic interactions such as dosage synthetic lethality, genetic suppression, and synthetic haplo-insufficiency. The protocol for synthetic lethality analysis with a conditional allele (*cdc102-1*) could also be used to study similar genetic interactions concerning specific alleles of any nonessential gene. In addition, this whole set of tools could be modified to study genome-wide synthetic interactions in response to chemicals or other physiological stresses.

One potential advantage of microarray-based functional profiling analysis is data quantitation, which has to some extent been hampered by low hybridization signal intensity for a subset of TAGs, the biggest technical weakness of the method. A good example is our benomyl-sensitivity screen, where all mutants of the prefoldin complex exhibited similar sensitivity to 5 μg/ml of benomyl in individual tests, but the hybridization C/E ratios in the screen are somewhat different from one another (Figure 3B). This was because some of the TAGs had low hybridization signal intensity, and these TAGs normally gave rise to low C/E ratios. This low hybridization intensity problem was caused by mutations in the TAGs or common primers (Eason et al., 2004). Redesign of the oligonucleotide microarray and hybridization protocols according to the actual TAG sequences found in the heterozygous diploid YKOs should further enhance the performance of dSLAM.

Practically, our methods are fast and cost-effective. Each biological sample is in a single petri dish or microfuge tube during the entire procedure, simplifying scale-up to the whole yeast genome, and decreasing the possibility of cross contamination between samples and variations within an experiment. Thus, it should be possible to map ~6000 × ~6000 genetic interactions of any kind in yeast using these techniques. In particular, ~6000 synthetic lethality screens ensure that each pair of interaction is screened bidirectionally with each gene in an interacting pair serving as query and target, further reducing false positive and negative rates. A comprehensive yeast genetic interaction map will guide further detailed dissection of the molecular network within a living yeast cell and other organisms.

Experimental Procedures

The Haploid Selection Marker and Query Constructs

LEU2 was PCR amplified (primers: 5′-TCGGATAAAACCAAAATAAG TACAAAGCCATCGAATAGAAATGTCTGCCCCTAAGAAGAT-3′ and 5′-TAAAGGAGGGAGAACAACGTTTTTGTACGCAGAAACTAAGCA AGGATTTTCTTAACTTCT-3′) and integrated 496 bp upstream to the *MFA1pr-HIS3* SGA reporter in strain Y2454 (Tong et al., 2001) via homologous recombination. The resulting *LEU2-MFA1pr-HIS3* cassette was PCR amplified (primers: 5′-GACTTCTTAACTCCTGTAAAA ACAAAAAAAAAAAAAAGGCATAGCACCTCAACATAACGAGAACAC-3′ and 5′-ATGGCGTGGAAATGTGATCAAAGGTAATAAAACGTCATA TCTACATAAGAACACCTTTGG-3′) and used to replace the *CAN1* ORF in the wild-type diploid yeast strain BY4743a/α (Brachmann et al., 1998) by selecting for Leu^+ transformants on SC-Leu medium. Tetrad analysis was performed to ensure that Leu^+ is linked to canavanine-resistance (Can^R) and that the *HIS3* reporter is expressed only in haploid *MAT***a** cells. A *CAN1L-LEU2-MFA1pr-HIS3-CAN1R* cassette that contains ~1 kb flanking sequence of the *CAN1* locus was amplified (primers: 5′-GGAACACGGAGTAAAATATTG-3′ and 5′-CATCCACCAAATGCAAATAAC-3′) from a Leu^+ Can^R His^+ *MAT***a** haploid strain (XPY433a) and cloned into the pCR2.1 vector via TA cloning and retested for functionality, forming pXP346.

A *kanMX* module in the existing YKO strains was replaced with a *loxP-URA3-loxP* gene (pSO142-4; S.L. Ooi and J.D.B., unpublished data) or a *natMX* module (Goldstein and McCusker, 1999) via homologous recombination to create query mutations used in the synthetic interaction and suppression studies. All query mutation constructs were PCR amplified (Takara Extaq, Panvera) and contained 1.5 kb flanking sequences for high efficiency integrative transformation into a pool of heterozygous diploid YKOs. Primers for amplifying the query constructs used are listed (Supplemental Table S9).

Plasmids used in the dosage synthetic lethality and suppression studies include YCplac33 (*URA3*, CEN), and pXP465 (*GAL1pr-LCD1*

in YCplac33) subcloned (with SalI/EcoRI) from pML260 (Paciotti et al., 2000). The plasmid pXP350 used in the *cdc102-1* synthetic lethality screen contains the wide-type *CDC102* on YCplac33. pXP362 (YCplac33-*cdc102-1*) contains a thermosensitive allele of *CDC102* that is partially functional at 28°C and 30°C but not functional at 37°C.

Yeast YKO Pool Construction

Each of a collection of 5996 *MAT***a**/α heterozygous diploid YKOs (Research Genetics) was tested for His^+ phenotype and mating type. 87 either contained the wild-type *HIS3* gene when constructed or behaved like haploid strains in a mating type test and were excluded from the pool. The strains on plates 280 and 281 (Research Genetics) were also omitted for experimental convenience because most of these were mating proficient. 23 of the omitted strains are essential gene YKOs (Supplemental Table S3). The remaining 5896 heterozygous diploid YKO strains were used to construct an original pool as previously described (Ooi et al., 2001). 10 μg pXP346 plasmid that contains the *CAN1L-LEU2-MFA1pr-HIS3-CAN1R* cassette was transformed en masse after double digestion with SpeI and PstI into this pool. A total of about 1.2×10^6 Leu^+ transformants were combined to make the haploid-convertible pool. 1 or 2 ml aliquots of this pool were frozen in 15% glycerol at 75 OD_{600}/ml (Figure 1A).

En Masse Transformation

For each transformation, an aliquot of the haploid-convertible heterozygous diploid YKOs was inoculated in 50 ml YPD liquid at 0.125 OD_{600}/ml and shaken at 30°C for 5.5 hr to an OD of ~0.5 OD_{600}/ml. The culture was harvested, washed once in 10 ml of water and subsequently in 10 ml of 0.1 M lithium acetate (LiOAc), and resuspended in residual 0.1 M LiOAc in a total volume of 100 μl. A transformation mixture of the following composition was freshly made: 480 μl 50% polyethylene glycol (PEG-3350, sigma), 72 μl 1 M LiOAc, 40 μl sheared, heat-denatured herring sperm DNA (10 mg/ml), and 28 μl transforming DNA solution. This transformation mixture was added to and mixed well with the 100 μl of yeast competent cells and incubated at 30°C for 30 min. 72 μl of dimethyl sulfoxide (DMSO) was then added to the transformation reaction, mixed thoroughly, and heat-shocked at 42°C for 13 min. Cells were spun down at 3600 rpm for 30 s, resuspended, and incubated in 1 ml 5 mM $CaCl_2$ for 5–15 min at room temperature. All cells from one transformation were spread on one 150 × 25 mm petri dish containing the appropriate selection medium (SC-Ura for *URA3* selection, SC-Leu for *LEU2* selection, and YPD plus 50 μg/ml of CloNat for *natMX* selection) and incubated at 30°C for 2 days. Dilution of the transformation was also spread on the selection medium for calculation of transformation yields. 10–20 μg of transforming DNA was used for each integrative transformation and ~5 μg for each plasmid transformation, obtaining $\sim 10^6$ independent transformants that are necessary to prevent stochastic loss of individual YKOs from the pool (Ooi et al., 2001). Ura^+ transformants from the *tub1*Δ*::URA3* transformation were directly analyzed for synthetic haplo-insufficiency with a *ura3*Δ*::URA3* transformation as the control.

Regeneration of a Haploid YKO Pool

25 OD_{600} of each heterozygous diploid YKO pool was inoculated in 50 ml fresh YPD liquid and shaken at 30°C for 3 hr. Cells were harvested, washed in 25 ml of sterile water, and inoculated in 50 ml of liquid sporulation medium (1% of potassium acetate, 0.005% zinc acetate). Sporulation was performed by shaking that culture at 30°C for 5 days. 2–4 OD_{600} of sporulated culture was evenly spread on one 150 × 25 mm petri dish containing the appropriate haploid selection medium and incubated at 30°C for 2 days.

The basic synthetic haploid selection magic medium (MM, SC−Leu−His−Arg+Canavanine+G418) was used to select for haploid *MAT***a** YKOs from sporulated cultures of heterozygous diploid pools by exploiting the SGA reporter and modified for some of the other experiments as indicated below. One liter of this medium contains dextrose or galactose (20 g), Difco yeast nitrogen base without amino acids and ammonium sulfate (1.7 g), SC−Leu−His−Arg dropout mix (2 g), sodium glutamate (1 g), G418 (200 mg), and L-Canavanine (60 mg). Benomyl was added to this medium as indicated to select for haploid pools in the benomyl-sensitive experiments.

For synthetic interaction analysis with *cin8*Δ*::URA3*, *bim1*Δ*::URA3*, *sgs1*Δ*::URA3*, or *sml1*Δ*::URA3* knockout cassettes as the query mutations, uracil was also omitted from the haploid selection medium (MM−Ura) to select for haploid double YKOs (experimental pools). 0.1% of 5-FOA was added (MM+5FOA) to select for haploid single YKOs from the same sporulated cultures (control pools).

In the synthetic interaction screen with *cdc102-1* as the query mutation, the plasmid-harboring heterozygous double deletion mutant pool was sporulated and germinated on haploid selection medium lacking uracil (MM−Ura) to select for a pool that contains both single and double haploid YKOs as the control. 50 μg/ml of CloNat (Nourseothricin) was added to this haploid selection medium (MM−Ura+Nat) to select only for the double YKOs (experimental pool). In these double YKOs, the plasmid alleles of *CDC102* provide viability because the chromosomal copy of the gene was disrupted by the *natMX* Nourseothricin-resistant cassette. Selection for both the control and experimental pools at semipermissive temperatures (28°C and 30°C) allowed screening for synthetic interactions with the *cdc102-1* allele.

In dosage synthetic lethality and suppression experiments, haploid pools harboring plasmids YCplac33 (vector; *CEN4*, *URA3*) and Ycplac33-*GAL1pr-LCD1* (pXP465) were selected on MM with 2% galactose as the sole carbon source and incubated at 30°C for 3 days. The experimental *LCD1*-overexpressing pool was compared to a vector plasmid (YCplac33) pool to analyze the effect of *LCD1* overexpression on viability of each YKO.

Genomic DNA Preparation and Microarray Hybridization

Genomic DNA isolation was performed essentially as described (Ooi et al., 2001). Cy3- (for experiments) and Cy5-labeled (for control) UPTAG and DOWNTAG primers used to PCR amplify the probes for microarray hybridization are also as described (Ooi et al., 2001). Both Cy3- and Cy5-labeled probes were generated with an asymmetric PCR protocol (D.S.Y. and J.S.B., unpublished data), hybridized to a TAG microarray (Rosetta or Agilent), and washed as described (Ooi et al., 2001). 1 mM DTT was added to the hybridization and washing solutions to prevent oxidation and degradation of the Cy5-labeled probes. Each chip was washed with washing solution immediately before scanning with a GenePix 4000B scanner (Axon Instruments).

Microarray Data Analysis

Tiff image files of each microarray experiment were analyzed by using Imagene 5.1 and GeneSight 3.0 software (Biodiscovery) or Genepix 4.0. The median raw signal intensity for each hybridization feature was collected for analysis. Local background was subtracted, and the signal intensity was normalized according to the total signal intensity. A (Cy5/Cy3) C/E ratio was obtained for each feature. Only features with a background-subtracted signal intensity median ≥400 (~3-fold over background signal intensity) were further analyzed. TAGs giving rise to systematic false positive results across multiple experiments were discarded from further analysis. Hybridization C/E ratios between the UP- and DOWNTAG for each YKO with intensity values ≥400 were averaged. For mutations with hybridization intensity ≥400 from only one of two TAGs, the C/E ratio of that TAG was used without averaging.

Individual Confirmation of the Gene-Gene Interactions

The *cin8*Δ*::URA3, bim1*Δ*::URA3*, *sgs1*Δ*::URA3*, or *sml1*Δ*::URA3* query construct was transformed into individual heterozygous diploid YKOs pretransformed with the SGA reporter. Two independent Ura^+ transformants from each transformation were sporulated and analyzed by random spore analysis (Ooi et al., 2003), or at least one of them was analyzed by tetrad analysis for synthetic interaction between the query and target mutations. For confirmation of the dosage synthetic interaction caused by *LCD1* overexpression, a control plasmid (YCplac33) and pXP465 (*GAL1pr-LCD1*) were transformed into individual heterozygous diploid YKOs containing the SGA reporter. Two independent Ura^+ transformants were sporulated and spotted at 10× serial dilutions on haploid selection medium with 2% galactose as the sole carbon source. Growth of each *LCD1*-overexpressing strain was compared to the isogenic strain carrying the control plasmid. To confirm the synthetic haplo-insufficiency interactions with *TUB1*/*tub1*Δ, individual heterozygous diploid YKOs

were independently transformed with a *tub1Δ::URA3* and a *ura3Δ::URA3* query mutations. Growth of the resultant Ura^+ transformants from these two transformations was compared.

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Accession Numbers

All microarray data supporting this publication have been deposited at GEO (accession number: GSE1838).