

## Synthetic Genetic Array Analysis in *Saccharomyces cerevisiae*

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### Summary

Synthetic lethality occurs when the combination of two mutations leads to an inviable organism. Screens for synthetic lethal genetic interactions have been used extensively to identify genes whose products buffer one another or impinge on the same essential pathway. For the yeast *Saccharomyces cerevisiae*, we developed a method termed Synthetic Genetic Array (SGA) analysis, which offers an efficient approach for the systematic construction of double mutants and enables a global analysis of synthetic lethal genetic interactions. In a typical SGA screen, a query mutation is crossed to an ordered array of approx 5000 viable gene deletion mutants (representing ~80% of all yeast genes) such that meiotic progeny harboring both mutations can be scored for fitness defects. This array-based approach automates yeast genetic analysis in general and can be easily adapted for a number of different screens, including genetic suppression, plasmid shuffling, dosage lethality, or suppression.

**Key Words:** Yeast; genetics; synthetic lethal; SGA; deletion mutant; double mutant; genetic interaction network; suppression; plasmid shuffling; dosage lethality; dosage suppression.

### 1. Introduction

Genetic analysis is important for assessing the biological roles of genes in vivo and remains a powerful tool for identifying new components of specific pathways and for ordering the function of gene products within a pathway. A combination of mutations in two genes that results in death or reduced fitness is termed a synthetic lethal or synthetic sick interaction, respectively (*1*). Synthetic lethality has been used extensively in different model organisms to identify genes whose products buffer one another and impinge on the same essential process (*2–4*).

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For the budding yeast *Saccharomyces cerevisiae*, an international consortium of laboratories generated a collection of gene deletion mutants for each of the approx 6000 predicted genes, identifying approx 1000 essential genes and creating approx 5000 viable deletion mutants (5,6). The introduction of molecular tags or barcodes, a unique 20-bp DNA sequence at either end of the deletion cassette, identifies each gene deletion strain and enables the fitness of a particular mutant to be assessed within a population using a barcode microarray (7). The collection of approx 5000 viable deletion mutants provided the first opportunity for systematic genetic analysis in yeast and the potential for examining 12.5 million different double mutants for a synthetic lethal or sick phenotype.

Synthetic genetic array (SGA) analysis enables the systematic construction of double mutants (8,9), allowing large-scale mapping of synthetic genetic interactions. A typical SGA analysis involves crossing a query strain to the array of approx 5000 viable deletion mutants, and through a series of replica-pinning procedures, the double mutants are selected and scored for growth defects. Applying SGA analysis to 132 query mutations enabled us to generate a genetic interaction network containing approx 1000 genes and approx 4000 interactions, with functional information associated with the position and connectivity of a gene on the network.

The SGA methodology is quite versatile because any genetic element (or any number of genetic elements) marked by a selectable marker(s) can be manipulated similarly. In this regard, SGA methodology automates yeast genetics generally, such that specific alleles of genes, including point mutants and temperature-sensitive alleles, or plasmids can be crossed into any ordered array of strains providing systematic approaches to genetic suppression analysis, dosage lethality, dosage suppression, or plasmid shuffling. In this chapter, we describe the steps of SGA analysis in detail and hope to encourage other laboratories to adopt this methodology to suit their specific fields.

## 2. Materials

### 2.1. Media and Stock Solutions

1. G418 (Geneticin, Invitrogen): Dissolve in water at 200 mg/mL, filter-sterilize, and store in aliquots at 4°C.
2. clonNAT (nourseothricin, Werner BioAgents, Jena, Germany): Dissolve in water at 100 mg/mL, filter-sterilize, and store in aliquots at 4°C.
3. Canavanine (L-canavanine sulfate salt; Sigma): Dissolve in water at 100 mg/mL, filter-sterilize, and store in aliquots at 4°C.
4. Thialysine (S-[2-aminoethyl]-L-cysteine hydrochloride; Sigma): Dissolve in water at 100 mg/mL, filter-sterilize, and store in aliquots at 4°C.
5. Amino-acids supplement powder mixture for synthetic media (complete): Contains 3 g adenine (Sigma), 2 g uracil (ICN), 2 g inositol, 0.2 g para-aminobenzoic

acid (Acros Organics), 2 g alanine, 2 g arginine, 2 g asparagine, 2 g aspartic acid, 2 g cysteine, 2 g glutamic acid, 2 g glutamine, 2 g glycine, 2 g histidine, 2 g isoleucine, 10 g leucine, 2 g lysine, 2 g methionine, 2 g phenylalanine, 2 g proline, 2 g serine, 2 g threonine, 2 g tryptophan, 2 g tyrosine, 2 g valine (Fisher). Drop-out (DO) powder mixture is a combination of the aforementioned ingredients minus the appropriate supplement. 2 g of the DO powder mixture is used per liter of medium (*see Note 1*).

6. Amino-acids supplement for sporulation medium: Contains 2 g histidine, 10 g leucine, 2 g lysine, 2 g uracil; 0.1 g of the amino-acid supplements powder mixture is used per liter of sporulation medium (*see Note 1*).
7.  $\beta$ -glucuronidase (Sigma): Prepare 0.5% solution in water and store at 4°C.
8. Glucose (Dextrose, Fisher): Prepare 40% solution, autoclave, and store at room temperature.
9. YEPD: Add 120 mg adenine (Sigma), 10 g yeast extract, 20 g peptone, 20 g bacto agar (BD Difco) to 950 mL water in a 2-L flask. After autoclaving, add 50 mL of 40% glucose solution, mix thoroughly, cool to approx 65°C, and pour plates.
10. YEPD + G418: Cool YEPD medium to approx 65°C, add 1 mL of G418 stock solution (final concentration 200 mg/L), mix thoroughly, and pour plates.
11. YEPD + clonNAT: Cool YEPD medium to approx 65°C, add 1 mL of clonNAT stock solution (final concentration 100 mg/L), mix thoroughly, and pour plates.
12. YEPD + G418/clonNAT: Cool YEPD medium to approx 65°C, add 1 mL of G418 (final concentration 200 mg/L), and 1 mL of clonNAT (final concentration 100 mg/L) stock solutions, mix thoroughly, and pour plates.
13. Enriched sporulation: Add 10 g potassium acetate (Fisher), 1 g yeast extract, 0.5 g glucose, 0.1 g amino-acids supplement powder mixture for sporulation, 20 g bacto agar to 1 L water in a 2-L flask. After autoclaving, cool medium to approx 65°C, add 250  $\mu$ L of G418 stock solution (final concentration 50 mg/L), mix thoroughly, and pour plates.
14. (SD/MSG) – His/Arg/Lys + canavanine/thialysine/G418: Add 1.7 g yeast nitrogen base without amino acids or ammonium sulfate (BD Difco), 1 g MSG (L-glutamic acid sodium salt hydrate; Sigma), 2 g amino-acids supplement powder mixture (DO – His/Arg/Lys), 100 mL water in a 250-mL flask. Add 20 g bacto agar to 850 mL water in a 2-L flask. Autoclave separately. Combine autoclaved solutions, add 50 mL 40% glucose, cool medium to approx 65°C, add 0.5 mL canavanine (50 mg/L), 0.5 mL thialysine (50 mg/L), and 1 mL G418 (200 mg/L) stock solutions, mix thoroughly, and pour plates (*see Note 2*).
15. (SD/MSG) – His/Arg/Lys + canavanine/thialysine/clonNAT: Add 1.7 g yeast nitrogen base without amino acids or ammonium sulfate, 1 g MSG, 2 g amino-acids supplement powder mixture (DO – His/Arg/Lys), 100 mL water in a 250-mL flask. Add 20 g bacto agar to 850 mL water in a 2-L flask. Autoclave separately. Combine autoclaved solutions, add 50 mL 40% glucose, cool medium to approx 65°C, add 0.5 mL canavanine (50 mg/L), 0.5 mL thialysine (50 mg/L), and 1 mL clonNAT (100 mg/L) stock solutions, mix thoroughly, and pour plates.
16. (SD/MSG) – His/Arg/Lys + canavanine/thialysine/G418/clonNAT: Add 1.7 g yeast nitrogen base without amino acids or ammonium sulfate, 1 g MSG, 2 g

amino-acids supplement powder mixture (DO – His/Arg/Lys), 100 mL water in a 250-mL flask. Add 20 g bacto agar to 850 mL water in a 2-L flask. Autoclave separately. Combine autoclaved solutions, add 50 mL 40% glucose, cool medium to approx 65°C, add 0.5 mL canavanine (50 mg/L), 0.5 mL thialysine (50 mg/L), 1 mL G418 (200 mg/L), and 1 mL clonNAT (100 mg/L) stock solutions, mix thoroughly, and pour plates.

17. (SD/MSG) Complete: Add 1.7 g yeast nitrogen base without amino acids or ammonium sulfate, 1 g MSG, 2 g amino-acids supplement powder mixture (complete), 100 mL water in a 250-mL flask. Add 20 g bacto agar to 850 mL water in a 2-L flask. Autoclave separately. Combine autoclaved solutions, add 50 mL of 40% glucose, mix thoroughly, cool medium to approx 65°C, and pour plates.
18. SD – His/Arg/Lys + canavanine/thialysine: Add 6.7 g yeast nitrogen base without amino acids (BD Difco), 2 g amino-acids supplement powder mixture (DO – His/Arg/Lys), 100 mL water in a 250-mL flask. Add 20 g bacto agar to 850 mL water in a 2-L flask. Autoclave separately. Combine autoclaved solutions, add 50 mL 40% glucose, cool medium to approx 65°C, add 0.5 mL canavanine (50 mg/L), and 0.5 mL thialysine (50 mg/L) stock solutions, mix thoroughly, and pour plates (*see Note 3*).
19. SD – Leu/Arg/Lys + canavanine/thialysine: Add 6.7 g yeast nitrogen base w/o amino acids, 2 g amino-acids supplement powder mixture (DO – Leu/Arg/Lys), 100 mL water in a 250-mL flask. Add 20 g bacto agar to 850 mL water in a 2-L flask. Autoclave separately. Combine autoclaved solutions, add 50 mL 40% glucose, cool medium to approx 65°C, add 0.5 mL canavanine (50 mg/L), and 0.5 mL thialysine (50 mg/L) stock solutions, mix thoroughly, and pour plates.

## 2.2. Plates and Accessories

1. OmniTrays (Nunc, cat. no. 242811) are used for SGA analysis (*see Note 4*).
2. 60-mm dishes (Fisher) are used for random spore analysis (*see Note 5*).
3. Aluminum sealing tape (Nunc, cat. no. 276014) is used for resealing the 96-well plates that contain the frozen stocks of yeast deletion strains.

## 2.3. Manual Pin Tools

The following manual pin tools can be purchased from V & P Scientific, Inc. (San Diego, CA).

1. 96 floating pin E-clip style manual replicator (VP408FH).
2. 384 floating pin E-clip style manual replicator (VP384F).
3. For extra floating pins (FP): 1.58 mm diameter with chamfered tip (*see Note 6*).
4. Registration accessories: Library Copier™ (VP381), Colony Copier™ (VP380).
5. Pin-cleaning accessories: plastic bleach or water reservoirs (VP421), pyrex alcohol reservoir with lid (VP420), pin-cleaning brush (VP425) (*see Note 7*).

## 2.4. Robotic Pinning Systems

1. VersArray colony arrayer system (BioRad Laboratories).
2. QBot, QPixXT, MegaPix (Genetix, Boston, MA).
3. Singer Rotor HDA bench top robot (Singer Instruments, Somerset, UK) (*see Note 4*).

## 2.5. Strains and Plasmids

1. Six different starting strains were constructed and used in the SGA screens (*see Table 1*). Y5563 (*MAT $\alpha$  can1 $\Delta$ ::MFA1pr-HIS3 lyp1 $\Delta$  ura3 $\Delta$ 0 leu2 $\Delta$ 0 his3 $\Delta$ 1 met15 $\Delta$ 0*) and Y5565 (*MAT $\alpha$  can1 $\Delta$ ::MFA1pr-HIS3 mfa1 $\Delta$ ::MF $\alpha$ 1pr-LEU2 lyp1 $\Delta$  ura3 $\Delta$ 0 leu2 $\Delta$ 0 his3 $\Delta$ 1 met15 $\Delta$ 0*) are the current starting strains for the construction of an SGA query strain (*see Note 8*).
2. p4339 (pCRII-TOPO::natRMX4; *see Note 9*).
3. Y7221 (*MAT $\alpha$  can1 $\Delta$ ::MFA1pr-HIS3 lyp1 $\Delta$  cyh2 ura3 $\Delta$ ::natR leu2 $\Delta$ 0 his3 $\Delta$ 1 met15 $\Delta$ 0*) is the wild-type control strain for the *natR*-marked query strains.
4. The collection of *MAT $\alpha$*  deletion strains can be purchased from Invitrogen (<http://www.invitrogen.com>) as stamped 96-well agar plates, American Type Culture Collection (<http://www.atcc.org/cydac/cydac.cfm>) as stamped 96-well agar plates, EUROSCARF (<http://www.uni-frankfurt.de/fb15/mikro/euroscarf/index.html>) as stamped 96-well agar plates, and Open Biosystems ([http://www.openbiosystems.com/yeast\\_collections.php](http://www.openbiosystems.com/yeast_collections.php)) as stamped 96-well agar plates or frozen stocks in 96-well plates.

## 3. Methods

### 3.1. SGA Query Strain Construction

#### 3.1.1. Nonessential Genes: PCR-Mediated Gene Deletion

1. Two gene-deletion primers are synthesized, each containing 55 bp of sequence at the 5' end that is specific to the region upstream or downstream of the gene of interest (*Gene X*), excluding the start and stop codons, and 22 bp of sequence at the 3' end that is specific for the amplification of the *natMX4* (**10**) cassette (**Fig. 1A** and **Table 2**).
2. The *natMX4* cassette flanked with 55 bp target sequences is amplified from p4339 with the gene-deletion primers designed in **step 1** (*see Note 10*).
3. Transform the polymerase chain reaction (PCR) product into the SGA starting strain, Y5563. Select transformants on YEPD + clonNAT medium.
4. Verify correct targeting of the deletion cassette by PCR.

#### 3.1.2. Nonessential Genes: Switching Method

1. Obtain the deletion strain of interest (*xxx $\Delta$ ::kanR*) from the *MAT $\alpha$*  deletion collection and mate with Y5565, isolate diploid zygotes by micromanipulation (**Fig. 2**).

**Table 1**  
**Yeast Strains**

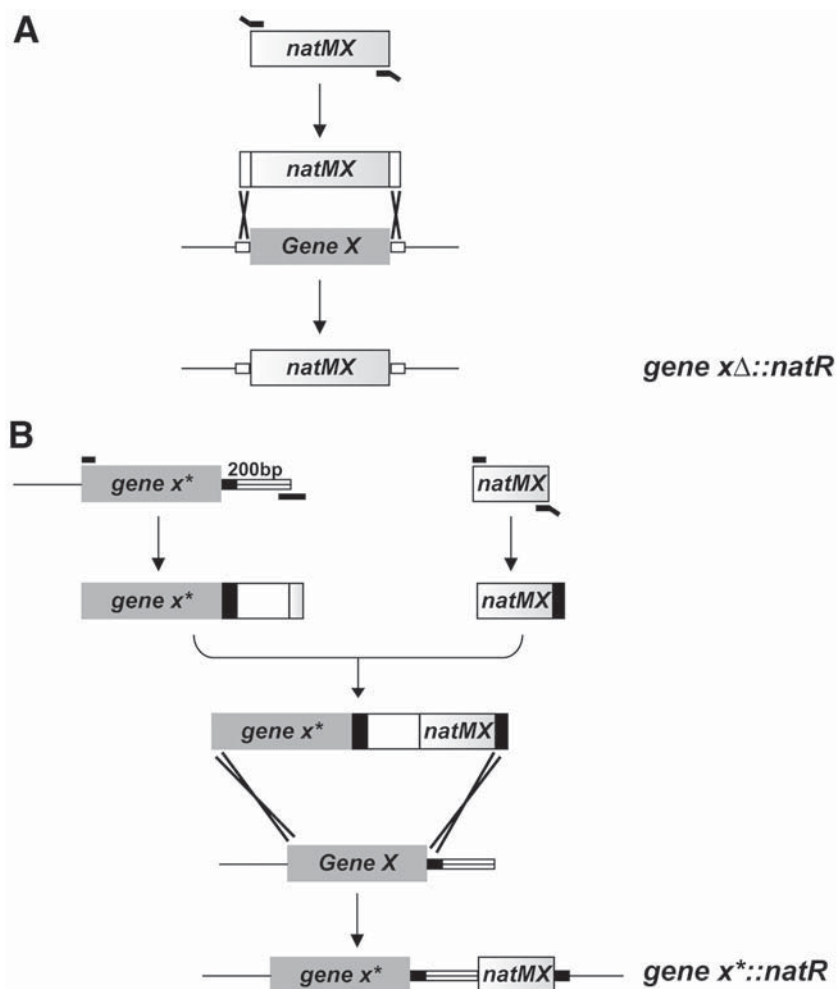
Strain	Genotype	Source
Y2454	<i>MATα mfa1Δ::MFA1pr-HIS3 can1Δ ura3Δ0 leu2Δ0 his3Δ1 lys2Δ0</i>	ref. 8
Y3068	<i>MATα can1Δ::MFA1pr-HIS3 ura3Δ0 leu2Δ0 his3Δ1 met15Δ0 lys2Δ0</i>	ref. 8
Y3084	<i>MATα can1Δ::MFA1pr-HIS3 mfa1Δ::MFα1pr-LEU2 ura3Δ0 leu2Δ0 his3Δ1 met15Δ0 lys2Δ0</i>	ref. 9
Y3656	<i>MATα can1Δ::MFA1pr-HIS3-MFα1pr-LEU2 ura3Δ0 leu2Δ0 his3Δ1 met15Δ0 lys2Δ0</i>	ref. 9
Y5563	<i>MATα can1Δ::MFA1pr-HIS3 lyp1Δ ura3Δ0 leu2Δ0 his3Δ1 met15Δ0</i>	Boone Lab
Y5565	<i>MATα can1Δ::MFA1pr-HIS3 mfa1Δ::MFα1pr-LEU2 lyp1Δ ura3Δ0 leu2Δ0 his3Δ1 met15Δ0</i>	Boone Lab
Y7221	<i>MATα can1Δ::MFA1pr-HIS3 lyp1Δ ura3Δ0::natR leu2Δ0 his3Δ1 met15Δ0 cyh2</i>	Boone Lab

2. Transform the resulting diploid with *EcoRI*-cut p4339, which switches the gene deletion marker from *kanMX* to *natMX*. Select transformants on YEPD + clonNAT medium.
3. Transfer the resultant diploids to enriched sporulation medium, incubate at 22°C for 5 d.
4. Resuspend a small amount of spores in sterile water, and plate on SD – Leu/Arg/Lys + canavanine/thialysine to select *MATα* meiotic progeny; incubate at 30°C for approx 2 d (see **Note 11**).
5. Replica plate to YEPD + clonNAT to identify the *MATα* meiotic progeny that carry the query deletion marked with *natMX* (*xxxΔ::natR*).

3.1.3. Essential Genes: PCR-Mediated Integration of Conditional Allele

1. Two pairs of oligonucleotides are synthesized. The first pair of primers is used in the amplification of the conditional allele of interest (*gene x\**), including 200 bp downstream of its stop codon, such that the reverse primer contains an additional 25 bp complementary sequence to the *natMX4* cassette at the 5' end (**Fig. 1B**). The second pair of primers is used in the amplification of the *natMX4* cassette, such that the reverse primer contains a 45 bp complementary sequence downstream of the target gene (*Gene X*).

**Fig. 1.** (opposite page) Strategies of construction of the SGA query strain. (A) PCR-mediated gene deletion is used to construct a nonessential query strain. The lines outside of the boxes represent the primers used for the PCR reaction. The thicker lines represent the primer sequences that anneal to the *natMX4* cassette (see **Table 2**). The thinner lines represent the 55 bp sequence specific to the upstream or downstream sequences of the target gene (*Gene X*). The *natMX4* cassette with flanking sequences is amplified and



**Fig. 1. (continued)** transformed into the SGA starting strain, Y5563. Gene deletion is mediated by homologous recombination between the ends of the target sequences of the PCR product and the corresponding genomic DNA sequence. Transformants carrying the target gene deletion (*gene x $\Delta$ ::natR*) are selected on YEPD + clonNAT. **(B)** Two-step PCR-mediated gene integration is used to construct a conditional allele query strain. First, the conditional allele and the marker are amplified separately. The conditional allele of interest (*gene x\**) and 200 bp downstream of its stop codon is amplified using primers to create a fragment that overlaps with the *natMX4* cassette. The *natMX4* cassette is amplified using primers to create a fragment that overlaps with the immediate downstream sequence of the target gene. Second, the PCR products are combined and co-transformed into the SGA starting strain, Y5563. Transformants are selected on YEPD + clonNAT under the permissive condition. Correct integration of the conditional allele (*gene x\*::natR*) is identified by replica plating to the restrictive condition.

**Table 2**  
**Primer Sequences**

Primer	Sequence 5' to 3'	Comments
MX-F	ACATGGAGGCCCGAGAATACCCT	MX-cassette amplification
MX-R	CAGTATAGCGACCAGCATTAC	MX-cassette amplification

2. Mix the two PCR products together and transform into the SGA starting strain, Y5563. Select transformants on YEPD + clonNAT medium.
3. Verify correct targeting of the conditional allele by replica plating to the restrictive condition.

**3.2. Sterilization Procedure for the Pin Tools**

*3.2.1. Manual Pin Tools*

1. Set up the wash reservoirs as follows: three trays of sterile water of increasing volume: 30 mL, 50 mL, and 70 mL, one tray of 40 mL of 10% bleach, one tray of 90 mL of 95% ethanol (*see Note 12*).
2. Let the replicator sit in the 30-mL water reservoir for approx 1 min to remove the cells on the pins.
3. Place the replicator in 10% bleach for approx 20 s.
4. Transfer the replicator to the 50-mL water reservoir and then to the 70-mL water reservoir to rinse the bleach off the pins.
5. Transfer the replicator to 95% ethanol.
6. Let excess ethanol drip off the pins, then flame.
7. Allow replicator to cool (*see Note 13*).

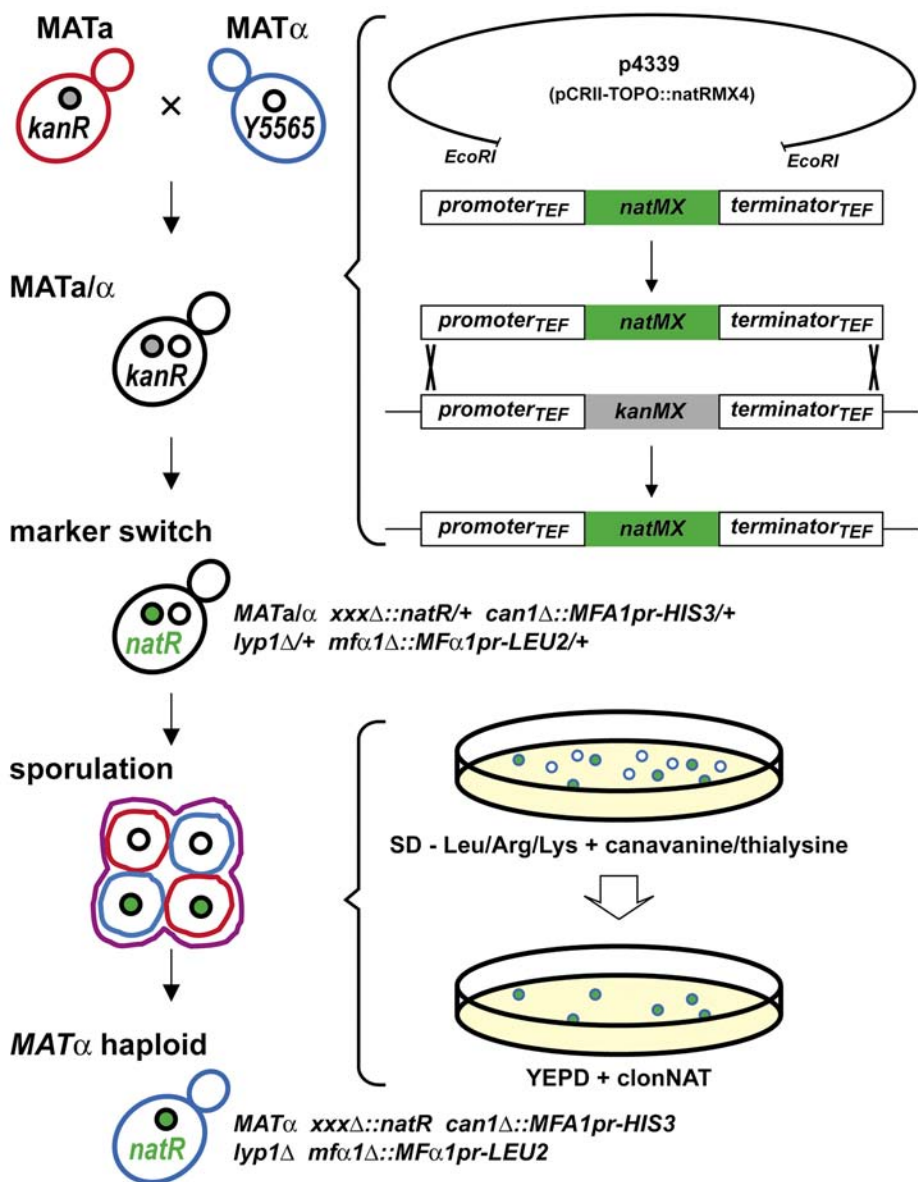
*3.2.2. Robotic Pin Tools (VersArray colony arrayer system)*

Use the following procedure to clean and sterilize the pins prior to starting on the robot.

1. Fill the sonicator with 230 mL of sterile water.
2. Clean the replicator in the sonicator for 5 min.
3. Remove the water, fill the sonicator with 230 mL of 75% ethanol.
4. Sterilize the replicator in the sonicator for 5 min.
5. Let the replicator sit in a tray of 90 mL of 95% ethanol for 30 s.
6. Allow the replicator to dry over the fan for 30 s.

**Fig. 2.** (*opposite page*) Construction of the SGA query strain using the switching method. The *MATa* deletion strain of interest (*xxxΔ::kanR*) is crossed to the *MATα* switcher strain, Y5565. The resultant diploid is transformed with *EcoRI*-cut p4339 to switch the gene deletion marker from *kanMX* to *natMX*. The resultant diploid is transferred to medium with





**Fig. 2.** (continued) reduced levels of carbon and nitrogen to induce sporulation and the formation of haploid meiotic spore progeny. Spores are transferred to synthetic medium lacking leucine, which allows for selective germination of *MATα* meiotic progeny because only these cells express the *MFα1pr-LEU2* reporter; and containing canavanine and thialysine, which allows for selective germination of meiotic progeny that carries the *can1Δ* (*can1Δ::MFA1pr-HIS3*) and *lyp1Δ* markers. The *MATα* meiotic progeny are then replica plated to medium that contains clonNAT, which selects for growth of meiotic progeny that carries the gene deletion mutation (*xxxΔ::natR*).

Use the following procedure to sterilize the pins at the end of each replicating step.

1. Set up the wash reservoirs as follows: two trays of sterile water of 50 mL and 60 mL, respectively, a tray of 90 mL of 95% ethanol, and the sonicator with 230 mL of 75% ethanol.
2. Let the replicator sit in the 50-mL water reservoir for 1 min to remove the cells on the pins.
3. Let the replicator sit in the 60-mL water reservoir for 1 min to remove the cells on the pins.
4. Sterilize the replicator in the 70% ethanol-sonicator for 2 min.
5. Let the replicator sit in the 95% ethanol for 30 s.
6. Allow the replicator to dry over the fan, for 30 s.

### **3.3. Building a 768-Density Deletion Mutant Array Using the Manual Pin Tools**

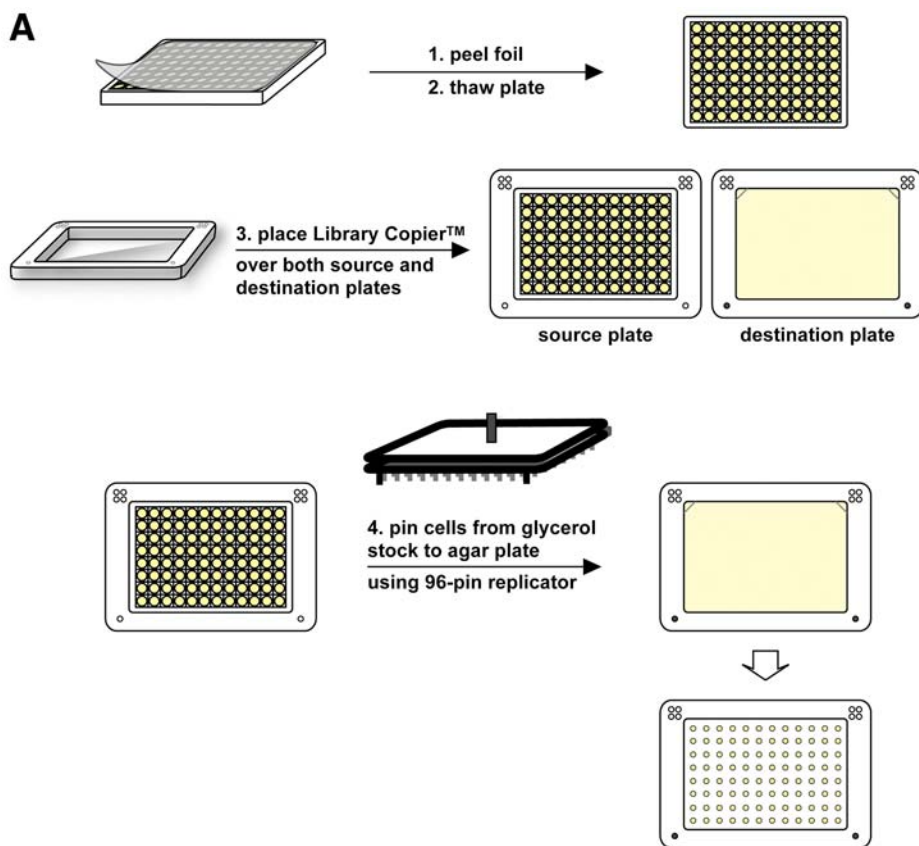
1. Peel off the foil coverings slowly on the frozen 96-well microtiter plates.
2. Let the plates thaw completely on a flat surface.
3. Mix the glycerol stocks gently by stirring with a 96-pin replicator.
4. Replicate the glycerol stocks from the 96-well plates onto YEPD + G418 agar plates using the Library Copier™ with the pair of one-alignment holes on the front frame (**Fig. 3A**). Take extreme caution that the pins do not drip liquid into neighboring wells.
5. Reseal the 96-well plates with fresh aluminum sealing tape and return to  $-80^{\circ}\text{C}$ .
6. Let cells grow at room temperature for approx 2 d.
7. Condense four plates of 96-format into one plate of 384-format using the 96-pin replicator and the Library Copier with the pair of four-alignment holes on the front frame (**Fig. 3B**).
8. Let cells grow at room temperature for approx 2 d (*see Note 14*).
9. Replicate the 384 strains onto a fresh plate with a 384-pin replicator and the Library Copier with the pair of four-alignment holes on the front frame. Use alignment holes “A” and “D” to create the working density of 768 (i.e., duplicates of 384 mutants).
10. Let cells grow at room temperature for approx 2 d, to generate the working copy of the deletion mutant array (DMA).

### **3.4. SGA Analysis**

#### **3.4.1. SGA Procedure**

**Figure 4** shows the selection steps in the SGA analysis. Query Strain and DMA.

1. Grow the query strain in a 5-mL overnight culture in YEPD.
2. Pour the query strain culture over a YEPD plate, use the replicator to transfer the liquid culture onto two fresh YEPD plates, generating a source of newly grown



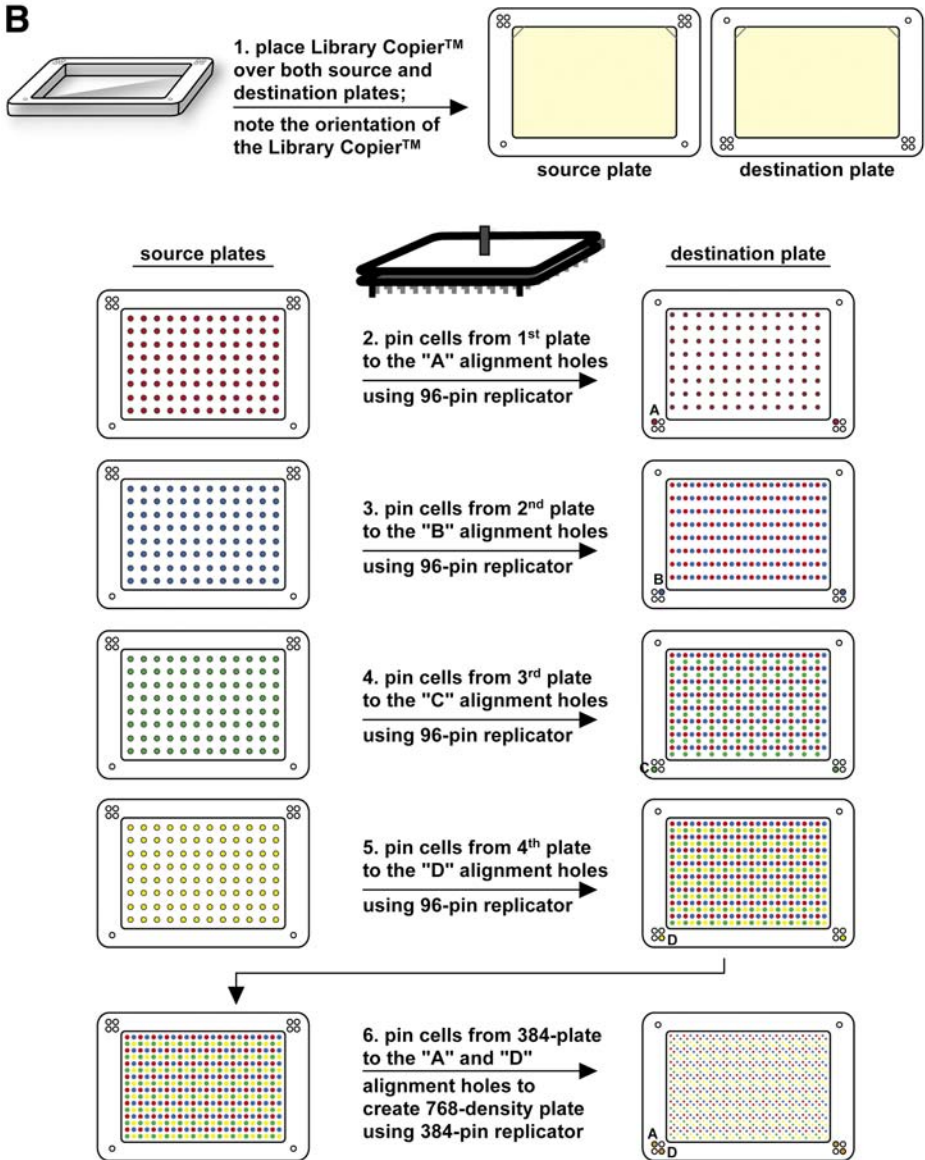
**Fig. 3.** Building a 768-density deletion mutant array (DMA) using the manual pin tools. Glycerol stocks are pinned to an agar plate using a 96-pin replicator and Library Copier with the pair of one-alignment holes on the front frame as depicted (**A**).

query cells for mating to the DMA in the density of 768. Let cells grow at 30°C for 1 d (*see* **Note 15**).

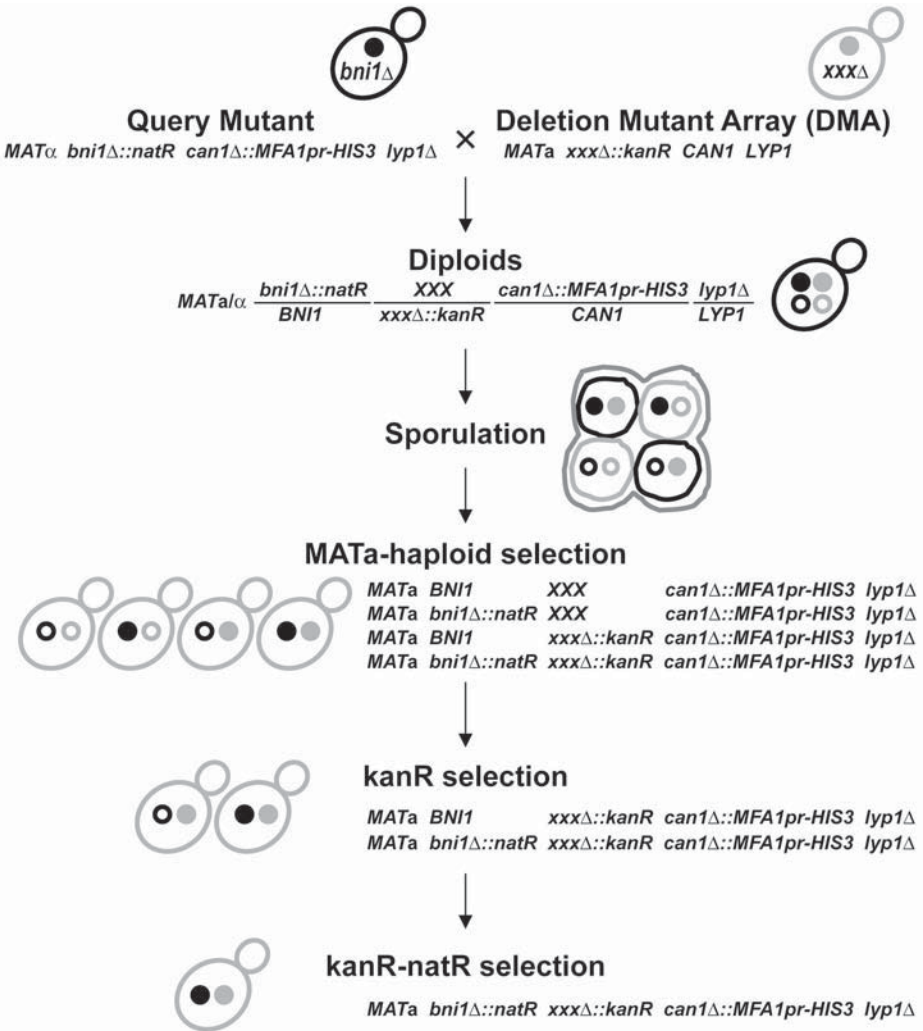
3. Replicate the DMA to fresh YEPD + G418. Let cells grow at 30°C for 1 d (*see* **Note 16**).

#### Mating the Query Strain with the DMA.

4. Pin the 768-format query strain onto a fresh YEPD plate.
5. Pin the DMA on top of the query cells.
6. Incubate the mating plates at room temperature for 1 d.



**Fig. 3.** Building a 768-density deletion mutant array (DMA) using the manual pin tools. Four 96-plates are condensed to form one 384-plate using the 96-pin replicator and Library Copier with the pair of four-alignment holes on the front frame as depicted (**B**). Finally, the 384 strains are transferred to a fresh plate using a 384-pin replicator and by registering the guide pins into alignment hole "A" of the Library Copier; this step is repeated but the guide pins are registered into alignment hole "D" of the Library Copier.



**Fig. 4.** Synthetic genetic array (SGA) methodology. A  $MAT_{\alpha}$  strain carrying a query mutation (*bni1* $\Delta$ ) linked to a dominant selectable marker, such as the nourseothricin-resistance marker *natMX* that confers resistance to the antibiotic nourseothricin (clonNAT), and the *MFA1pr-HIS3*, *can1* $\Delta$ , and *lyp1* $\Delta$  reporters is crossed to an ordered array of  $MAT_{\alpha}$  viable yeast deletion mutants, each carrying a gene deletion mutation linked to a kanamycin-resistance marker *kanMX* that confers resistance to the antibiotic geneticin (G418). Growth of resultant zygotes is selected for on medium containing nourseothricin and geneticin. The heterozygous diploids are transferred to medium with reduced levels of carbon and nitrogen to induce sporulation and the formation of haploid meiotic spore progeny. Spores are transferred to synthetic medium lacking histidine, which allows for selective germination of  $MAT_{\alpha}$  meiotic progeny

***MATa/α* Diploid Selection and Sporulation.**

7. Pin the resulting *MATa/α* zygotes onto YEPD + G418/clonNAT plates.
8. Incubate the diploid-selection plates at 30°C for 2 d.
9. Pin diploid cells to enriched sporulation medium.
10. Incubate the sporulation plates at 22°C for 5 d (*see Note 17*).

***MATa* Meiotic Progeny Selection.**

11. Pin spores onto SD – His/Arg/Lys + canavanine/thialysine plates.
12. Incubate the haploid-selection plates at 30°C for 2 d.
13. Pin the *MATa* meiotic progeny onto SD – His/Arg/Lys + canavanine, thialysine plates for a second round of haploid selection.
14. Incubate the plates at 30°C for 1 d.

***MATa*-kanR Meiotic Progeny Selection.**

15. Pin the *MATa* meiotic progeny onto (SD/MSG) – His/Arg/Lys + canavanine/thialysine/G418 plates.
16. Incubate the kanR-selection plates at 30°C for 2 d.

***MATa*-kanR-natR Meiotic Progeny Selection.**

17. Pin the *MATa* meiotic progeny onto (SD/MSG) – His/Arg/Lys + canavanine/thialysine/G418/clonNAT plates.
18. Incubate the kanR/natR-selection plates at 30°C for 2 d.
19. Score double mutants for fitness defect (*see Note 18*).

**3.4.2. Scoring of Putative Interactions in an SGA Screen**

1. Perform an SGA screen using the “wild-type” control strain (Y7221) following the steps as described in **Subheading 3.4.1**.
2. Visually inspect the experimental plates by comparing to the wild-type control plates, taking note of the double mutant colonies that fail to grow, or appear smaller in size (*see Note 19*).
3. Record the potential hits in the first-round screening.
4. Repeat the screen two more times, for a total of three independent screens.
5. Record the potential hits for the second- and third-round screenings.

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**Fig. 4.** (*continued*) because only these cells express the *MFA1pr-HIS3* reporter; and containing canavanine and thialysine, which allows for selective germination of meiotic progeny that carries the *can1Δ* and *lyp1Δ* markers. The *MATa* meiotic progeny are then transferred to medium that contains G418, which selects for growth of meiotic progeny that carries the gene deletion mutation (*xxxΔ::kanR*). Finally, the *MATa* meiotic progeny are transferred to medium that contains both clonNAT and G418, which then selects for growth of double mutant (*bni1Δ::natR xxxΔ::kanR*).



6. Generate an unbiased set of putative interactions by including all those that appear two or three times in the three rounds of screening.
7. Generate a biased set of putative interactions by sorting the one-time hits according to the functional annotations such as Gene Ontology (GO) molecular function and biological process, and selecting those that are related functionally to multiple genes within the unbiased set (*see Note 20*).
8. Combine both sets of putative interactions to create a list for confirmation.

### **3.5. Confirmation of the Putative Interactions Generated From SGA Analysis**

#### **3.5.1. Random Spore Analysis**

1. Inoculate a small amount of spores (approximately the size of a pinprick) in 1 mL of sterile water; mix well (*see Note 21*) (**11**).
2. Plate 20  $\mu$ L on SD – His/Arg/Lys + canavanine/thialysine (*see Note 22*).
3. Plate 40  $\mu$ L on (SD/MSG) – His/Arg/Lys + canavanine/thialysine/G418.
4. Plate 40  $\mu$ L on (SD/MSG) – His/Arg/Lys + canavanine/thialysine/clonNAT.
5. Plate 80  $\mu$ L on (SD/MSG) – His/Arg/Lys + canavanine/thialysine/G418/clonNAT.
6. Incubate the plates at 30°C for approx 1.5–2 d.
7. Score the double-drugs selection against the single-drug selections (**Fig. 5**).

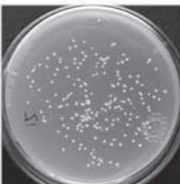
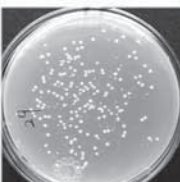
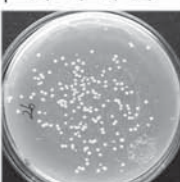
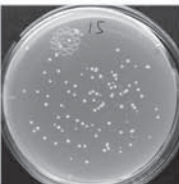
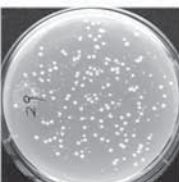
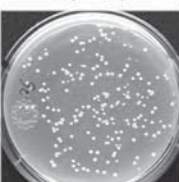
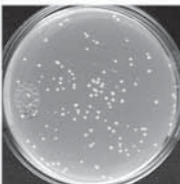
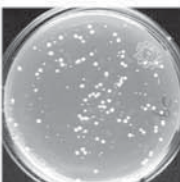
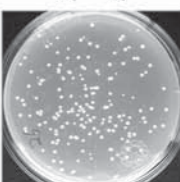
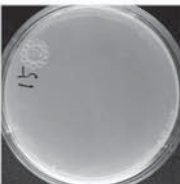
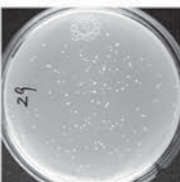
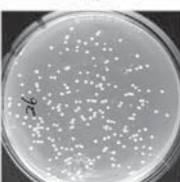
#### **3.5.2. Tetrad Analysis**

1. Inoculate a small amount of spores (approximately the size of a pinprick) in 100  $\mu$ L of 0.5%  $\beta$ -glucuronidase solution.
2. Mix gently by stirring the loop and incubate at room temperature for 15 min.
3. Spread approx 30  $\mu$ L of digested spores on (SD/MSG) Complete medium (*see Note 23*).
4. Dissect tetrads.

### **3.6. Applications of the SGA Methodology**

To examine synthetic genetic interactions with the essential genes, an SGA query strain can be crossed to the Tet-promoters Hughes collection (yTHC) (Open Biosystems), double mutants can be selected and scored for growth defects in the presence of doxycycline, which downregulates the expression of the essential genes (**12**).

The SGA methodology can be easily extended to other forms of genetic interactions, for example, higher-order genetic interactions (triple mutant genetic interactions) (**9**), dosage lethality, and suppression using high-copy plasmid or regulatory expression of yeast genes or heterologous genes. Reporter constructs, such as *SCB::HIS3* (**13**), can be incorporated into the SGA methodology to monitor specific transcriptional responses in the approx 5000 deletion mutant backgrounds. A Yeast Overexpression Array, containing

Selection	Genotype of the meiotic progeny on selection media			
	<b>A</b> <i>cog7Δ::kanR</i>	<b>B</b> <i>gos1Δ::kanR</i>	<b>C</b> <i>zrt1Δ::kanR</i>	
His, can, thi				MATa <i>can1Δ::MFA1pr-HIS3 lyp1Δ</i> , MATa <i>can1Δ::MFA1pr-HIS3 lyp1Δ arl1Δ::natR</i> , MATa <i>can1Δ::MFA1pr-HIS3 lyp1Δ xxxΔ::kanR</i> , and MATa <i>can1Δ::MFA1pr-HIS3 lyp1Δ arl1Δ::natR xxxΔ::kanR</i>
His, can, thi, clonNAT				MATa <i>can1Δ::MFA1pr-HIS3 lyp1Δ arl1Δ::natR</i> , and MATa <i>can1Δ::MFA1pr-HIS3 lyp1Δ arl1Δ::natR xxxΔ::kanR</i>
His, can, thi, G418				MATa <i>can1Δ::MFA1pr-HIS3 lyp1Δ xxxΔ::kanR</i> , and MATa <i>can1Δ::MFA1pr-HIS3 lyp1Δ arl1Δ::natR xxxΔ::kanR</i>
His, can, thi, G418, clonNAT				MATa <i>can1Δ::MFA1pr-HIS3 lyp1Δ arl1Δ::natR xxxΔ::kanR</i>
	SL	SS	No interaction	



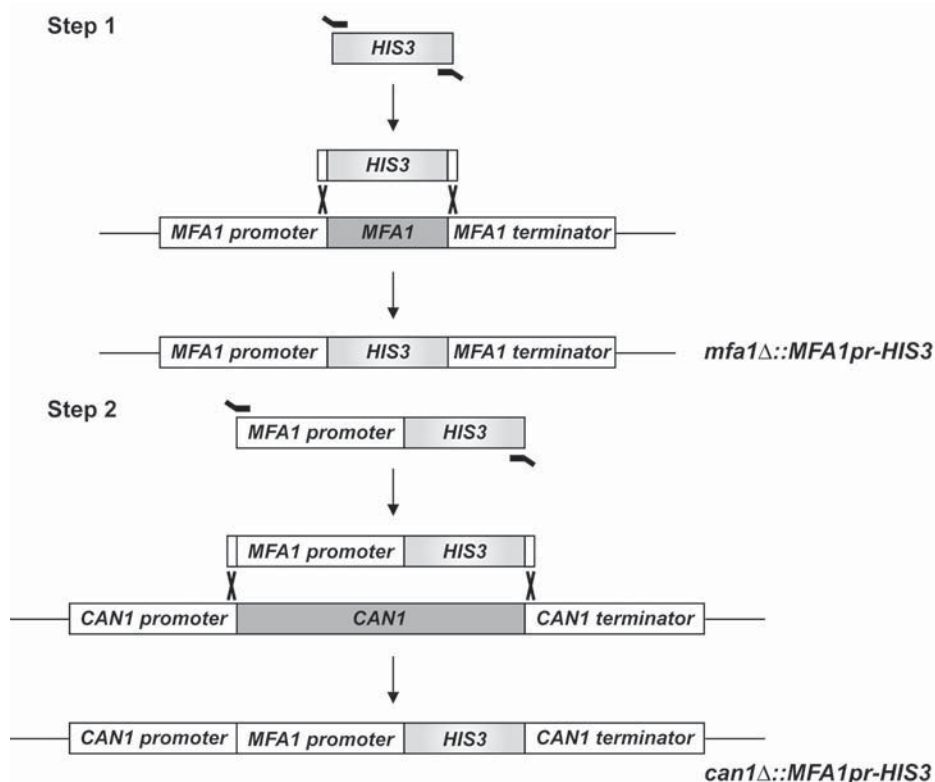
approx 6000 ORFs, has been assembled and can be used to screen for synthetic dosage lethality and suppression (R. Sopko, M. Snyder, C. Boone, and B. Andrews, unpublished data). Because double mutants are created by meiotic recombination, a set of gene deletions that is linked to the query gene, which we refer to as the “linkage group” form double mutants at a reduced frequency, thus, appearing synthetic lethal/sick with the query mutation. Because the gene deletions represent mapping markers covering all chromosomes in the yeast genome, SGA mapping (SGAM) has been shown as a method for high-resolution genetic mapping (14).

#### 4. Notes

1. When making up the amino acids supplement mixture, the solid ingredients should be combined and then mixed thoroughly by turning end-over-end for at least 15 min. The resultant mixture can be stored in tinted glass bottles at room temperature.
2. Because ammonium sulfate impedes the function of G418 and clonNAT, synthetic medium containing these antibiotics is made with monosodium glutamic acid as a nitrogen source (15).
3. Because this medium does not contain any antibiotics such as G418 and clonNAT, ammonium sulfate is used as the nitrogen source.
4. The Singer Rotor DHA bench top robot uses disposable replicators (RePads), and larger surface area plates that have the same external footprint dimensions as OmniTray, PlusPlates.
5. We use OmniTrays for all the replica pinning steps involved in SGA analysis, 100-mm petri dishes for the construction of SGA query strains and tetrad analysis, and 60-mm Petri dishes for random spore analysis. We found that approx 35 mL of media in an OmniTray gives the optimal result. Excess media might cause uneven transfer of cells during replica-pinning, such as the pins poking through the agar along the edges. For random spore analysis, approx 10 mL of media in a 60-mm dish is optimal.

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**Fig. 5.** (*opposite page*) Examples of the random spore analysis: *MATa* meiotic progeny derived from sporulation of heterozygous diploids, *MATa/α arl1Δ::natR/+ cog7Δ::kanR/+* (A), *MATa/α arl1Δ::natR/+ gos1Δ::kanR/+* (B), and *MATa/α arl1Δ::natR/+ zrt1Δ::kanR/+* (C), plated onto media (SD – His/Arg/Lys + canavanine/thialysine), ([SD/MSG] – His/Arg/Lys + canavanine/thialysine/clonNAT), ([SD/MSG] – His/Arg/Lys + canavanine/thialysine/G418), ([SD/MSG] – His/Arg/Lys + canavanine/thialysine/G418/clonNAT) as indicated. The plates were incubated at 30°C for approx 2 d. Cell growth under the four conditions was compared and scored. The *MATa arl1Δ::natR cog7Δ::kanR* double mutant (A) was scored as having a synthetic lethal (SL) interaction. The *MATa arl1Δ::natR gos1Δ::kanR* double mutant (B) was scored as having a synthetic sick (SS) interaction. The *MATa arl1Δ::natR zrt1Δ::kanR* double mutant (C) was scored as having no interaction (No).



**Fig. 6.** Construction of the starting strain. The construction of *can1Δ::MFA1pr-HIS3* involves two steps. First, the *HIS3* opening reading frame (ORF) is integrated at the *MFA1* locus such that its expression is regulated by the *MFA1* promoter (*MFA1pr*), *mfa1Δ::MFA1pr-HIS3*. Second, *mfa1Δ::MFA1pr-HIS3* is integrated at the *CAN1* locus, replacing the *CAN1* gene, *can1Δ::MFA1pr-HIS3*.

6. The 1.58-mm diameter, flat-tip pins (FP6) can be used as an alternative to the chamfered-tip pins. They transfer more cells than the chamfered-tip pins, and might not be suitable for producing high-density arrays (768 spots/array).
7. Empty tip boxes can be used as a substitute to the reservoirs for bleach, water, and ethanol.
8. In Y5565, *LEU2* was integrated at the *MF $\alpha$ 1* locus such that its expression is regulated by the *MF $\alpha$ 1pr* promoter (*MF $\alpha$ 1pr*), *mfa1Δ::MF $\alpha$ 1pr-LEU2*. In both Y5563 and Y5565, *MFA1pr-HIS3* was integrated at the *CAN1* locus, *can1Δ::MFA1pr-HIS3* (**Fig. 6**). In addition, they differ from the previous starting strains, because they carry a *lyp1* marker that confers resistance to thialysine. To create an SGA query strain by PCR-mediated integration or gene disruption, we use Y5563 (*MAT $\alpha$  can1Δ::MFA1pr-HIS3 lyp1Δ ura3Δ0 leu2Δ0 his3Δ1 met15Δ0*). To create an SGA query strain by the switching method, we use Y5565

(*MAT $\alpha$  can1 $\Delta$ ::MFA1pr-HIS3 mfa1 $\Delta$ ::MFA1pr-LEU2 lyp1 $\Delta$  ura3 $\Delta$ 0 leu2 $\Delta$ 0 his3 $\Delta$ 1 met15 $\Delta$ 0*).

9. Plasmid p4339 serves as a DNA template to amplify the *natRMX4* cassette required for PCR-mediated integration or gene deletion. It also serves as a *kanMX* to *natMX* maker-switcher plasmid.
10. Adding 5% dimethyl sulfoxide (DMSO) to the PCR reaction increases the product yield of the *natMX4* cassette.
11. To facilitate the selection of *MAT $\alpha$*  meiotic progeny that carries the query mutation by velvet-replica plating, we aim to plate approx 200–300 colonies on the SD – Leu/Arg/Lys + canavanine/thialysine medium.
12. To ensure the pins are cleaned properly and to avoid contamination in the wash procedure, the volume of wash liquids in the cleaning reservoirs is designed to cover the pins sequentially in small increments. For example, in the first step, only the tips of the pins should be submerged in water. As the pins are transferred through the cleaning reservoirs to the final ethanol step, the lower halves of the pins should be covered.
13. To reduce waiting time during the sterilization procedure, it is desirable to have three to four pinning tools such that they can be processed through the sterilization and pinning procedure in rotation.
14. To minimize contamination on the deletion mutant array (DMA), we propagate it on YEPD + G418 plates. This collection of 384-density plates should be maintained as the master plate set for SGA analysis and also as frozen stock at  $-80^{\circ}\text{C}$ . The agar plates can be kept at  $4^{\circ}\text{C}$  and propagated as needed, or revived from the frozen stock once every month.
15. Pinning the query strain in the 768-format on an agar plate is advantageous as cells are evenly transferred to the subsequent mating step. One query plate should contain a sufficient amount of cells for mating with eight plates of the DMA.
16. The DMA can be reused for three to four rounds of mating reactions.
17. It is important to keep the sporulation plates at approx  $22\text{--}24^{\circ}\text{C}$  for efficient sporulation. The resultant sporulation plates can be stored at  $4^{\circ}\text{C}$  for up to 4 mo without significant loss of spore viability, and provide a source of spores for random spore analysis and tetrad analysis.
18. The barcode microarrays can be used as an alternative method to score the double mutant for fitness defects. Because each of the deletion mutants is tagged with two unique oligonucleotide barcodes, their growth rates can be monitored within a population of cells. As shown in **Fig. 3**, the steps for creating double mutants can be carried out in pooled cultures and synthetic fitness defects can be analyzed using the barcode microarrays where the hybridization intensities reflect the representation of the double-mutant meiotic progeny. A technique called synthetic lethality analysis by microarray (SLAM) uses a transformation-based strategy to create a pool of double mutants, which can then be analyzed by the barcode microarrays (*II*).
19. In addition to visual inspection of the double mutants, we have developed a computer-based scoring system, which generates an estimate of relative growth rates

- from the area of individual colonies, as measured from digital images of the double-mutant plates. Statistical significance can be determined for each strain by comparing the measurements between the mutants and wild-type controls.
20. The programs FunSpec (<http://funspec.med.utoronto.ca/>) and FuncAssociate (<http://llama.med.harvard.edu/cgi/func/funcassociate>) are used to assign functional annotations in order to assist the sorting of putative interactions. FunSpec takes a list of genes as input and produces a summary of functional annotations from the MIPS and GO databases that are enriched in the list. FuncAssociate takes a list of genes as input and produces a ranked list of the GO annotations as enriched or depleted within the list.
  21. The spores are derived from the sporulation step in the SGA procedure. Alternatively, heterozygous diploids of the query mutation and test mutation can also be generated independently by mating the *MAT $\alpha$*  query strain to the *MATa* deletion strain of interest (*xxx $\Delta$ ::kanR*). The resulting diploids can then be induced for sporulation and used in the random spore analysis and tetrad analysis.
  22. The expected number of *MATa* meiotic progeny on each medium should be roughly equal. SD – His/Arg/Lys + canavanine/thialysine allows germination of the *MATa* meiotic progeny that carries the *can1 $\Delta$ ::MFA1pr-HIS3* and *lyp1 $\Delta$*  markers.  
 (SD/MSG) – His/Arg/Lys + canavanine/thialysine/G418 allows the germination of the *MATa* meiotic progeny that carries the *can1 $\Delta$ ::MFA1pr-HIS3* and *lyp1 $\Delta$*  markers, and the *kanR*-marked gene deletion.  
 (SD/MSG) – His/Arg/Lys + canavanine/thialysine/clonNAT allows the germination of the *MATa* meiotic progeny that carries the *can1 $\Delta$ ::MFA1pr-HIS3* and *lyp1 $\Delta$*  markers, and the *natR*-marked query mutation.  
 (SD/MSG) – His/Arg/Lys + canavanine/thialysine/G418/clonNAT allows the germination of the *MATa* meiotic progeny that carries the *can1 $\Delta$ ::MFA1pr-HIS3* and *lyp1 $\Delta$*  markers, and the double mutations of the *natR*-marked query and *kanR*-marked gene deletion.
  23. Because we cannot add the antibiotics (G418 and clonNAT) into the medium for tetrad analysis, the closest conditions to the double mutant selection step is synthetic dextrose (SD/MSG) Complete medium. This medium is more sensitive than the conventional rich medium in detecting subtle growth defects.

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