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16 High-Throughput Strain Construction and Systematic Synthetic Lethal Screening in *Saccharomyces cerevisiae*

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I. INTRODUCTION

Genetic analysis is a powerful way to assess gene function *in vivo*, identifying new components of specific pathways and ordering gene products within a pathway. Synthetic genetic interactions are usually identified when a second-site mutation, or increased gene dosage, suppresses or enhances the original mutant phenotype. This type of genetic screening approach has been used extensively in yeast, worms, flies, mice, and other model organisms. In particular, a genetic interaction termed ''synthetic lethality'' occurs when the combination of two otherwise viable mutations results in a lethal phenotype (Hartman *et al*., 2001; Kaelin, 2005). When two genes show a synthetic lethal interaction, it often reflects that the gene products impinge on the same essential function, such that one pathway functionally compensates for, or buffers, the defects in the other. Thus, large-scale mapping of genetic interactions should provide a global view of functional relationships between genes and pathways (Tong *et al*., 2004).

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In budding yeast *Saccharomyces cerevisiae*, a complete set of gene deletion mutants has been constructed for each of the ~6000 predicted genes in the genome, identifying ~1000 essential genes and creating ~5000 viable deletion mutants (Winzeler *et al*., 1999; Giaever *et al*., 2002). The fact that over 80% of the predicted genes are not required for life reflects the robustness of biological circuits and may reflect cellular buffering against genetic variation (Hartwell *et al*., 1999; Hartman *et al*., 2001; Hartwell, 2004). Hence, the collection of ~5000 viable deletion mutants represents a valuable resource for systematic genetic analysis, providing the potential to examine 12.5 million different double-mutant combinations for a synthetic lethal or sick phenotype. In this chapter, we focus on an array-based synthetic lethal analysis approach, termed synthetic genetic array (SGA) analysis (Tong *et al*., 2001, 2004), an automated method for constructing double mutants (or higher order allele combinations) and large-scale mapping of functional relationships among specific genes and pathways in yeast.

♦♦♦♦♦♦ II. IDENTIFICATION OF SYNTHETIC LETHAL INTERACTIONS

A. Classical Synthetic Lethal Screens

The availability of a haploid life cycle in yeast makes it particularly suitable for genetic analysis such as screens to identify synthetic lethal interactions. A classical synthetic lethal screen typically involves mutagenizing a strain carrying a mutation in a ''query'' gene of interest, and screening for mutants whose growth is dependent upon expression of the query gene, using a plasmid loss/colony-sectoring assay (Bender and Pringle, 1991). Subsequent identification of the synthetic lethal mutations requires complementation cloning with a plasmid-based genomic library. Although this approach has been used successfully to dissect genetic relationships among genes involved in cell polarity, secretion, DNA repair, transcription and many other biological processes, relatively few interactions are usually identified in a single screen (Bender and Pringle, 1991; Wang and Bretscher, 1997; Chen and Graham, 1998; Macpherson *et al*., 2000; Hartman *et al*., 2001; Mullen *et al*., 2001). Saturation is rarely achieved because the genetic analysis of the synthetic lethal double mutants and the subsequent cloning of the identified genes is time consuming.

B. Systematic Synthetic Lethal Screens – Synthetic Genetic Array (SGA) Analysis

We developed a method termed SGA analysis, which offers an efficient approach for the systematic construction of double mutants and enables a global analysis of synthetic lethal genetic interactions (Tong *et al*., 2001). A typical SGA screen involves crossing a query mutation to an ordered array of ~5000 viable gene deletion mutants, and, through a series of replica-pinning steps, meiotic progeny harboring both mutations can be recovered and scored for fitness defects (Figure 1, see Colour Plate section). This procedure can be performed using a colony pinning robot or manually using a hand-held replicator. Here, we outline the genetic logic underlying SGA analysis and describe the most recent version of SGA reagents and methodology. For additional information about the SGA system see Tong and Boone (2005).

1. SGA starting strains and media

(a) *A*-specific SGA reporters

The SGA methodology depends on the germination of *MAT***a** meiotic progeny, specifically, if both *MAT***a** and *MATα* meiotic progeny are germinated then haploid cells can mate with one another and generate diploids that are heterozygous for one or both deletion alleles, thereby leading to false negatives. To ensure the germination of a single mating type (Figure 1, Step 4), we linked a haploid mating-type specific promoter to a selectable marker. For example, the *MFA1* promoter (pr) sequence was fused with the *HIS3* open reading frame to create the SGA reporter *MFA1pr-HIS3*, which was then integrated at the *CAN1* locus (*can1Δ::MFA1pr-HIS3*) (Figure 2A). *MAT***a** cells carrying *MFA1pr-HIS3* are able to grow on medium lacking histidine, whereas *MATα* and *MAT***a**/*α* cells carrying *MFA1pr-HIS3* are unable to do so because the expression of *MFA1pr-HIS3* is repressed in these cells.

To investigate which **a**-specific promoter was most productive for SGA analysis, we created six different **a**-specific SGA reporters, derived from the **a**-specific genes listed in Table 1. Each reporter was constructed by fusing a different **a**-specific promoter sequence with the *HIS3* open reading frame, we then examined if appropriate expression of the *HIS3* gene occurs only in *MAT***a** cells but not *MATα* or *MAT***a**/*α* cells, using a selective growth assay on medium lacking histidine (SD-His). We found that all of the reporters showed mating-type specific expression as expected; however, *STE2pr-HIS3* was the most reliable in our experiments for two reasons. First, *MAT***a** cells carrying the reporter were His+ and grew at rates equivalent to that of *HIS3* cells on SD-His. This is in contrast to cells carrying the *ASG7pr-HIS3*, which showed a reduced fitness on SD-His. Second, the *STE2pr-HIS3* appeared to result in the lowest level of inappropriate expression of *HIS3* in *MATα* and *MAT***a**/*α* cells.

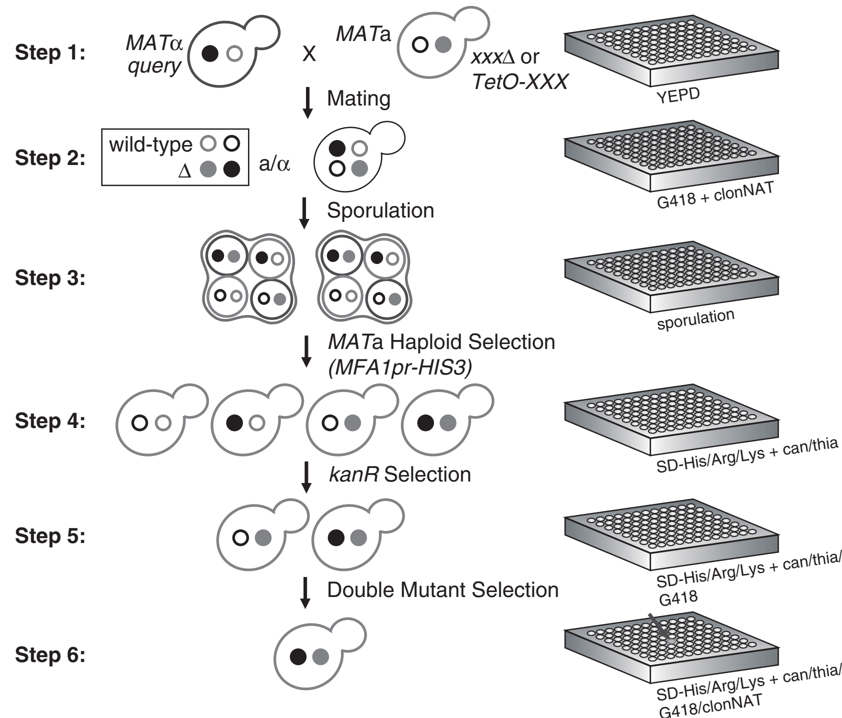

Figure 1. SGA methodology. Step 1, a *MATα* strain carrying a query mutation (*bni1Δ*) linked to a dominant selectable marker, such as the nourseothricin-resistance marker *natMX* that confers resistance to the antibiotics nourseothricin (clonNAT), and the *MFA1pr-HIS3*, *can1Δ* and *lyp1Δ* reporters is crossed to an ordered array of *MAT***a** viable deletion mutants (*xxxΔ*), each carrying a gene deletion mutation linked to a kanamycin-resistance marker *kanMX* that confers resistance to the antibiotic geneticin (G418). To score genetic interactions amongst essential genes, the query strain can be crossed to an array of conditional yeast mutants. For example, an array in which each mutant carries a different essential gene placed under the control of the conditional Tetracycline-regulated promoter (*TetO-XXX*); however, when screening the conditional array the selection conditions at each step differ from those outlined here as described previously (Mnaimneh *et al*., 2004; Davierwala *et al*., 2005). Step 2, growth of resultant zygotes is selected for on medium containing nourseothricin and geneticin. Step 3, 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. Step 4, spores are transferred to synthetic medium lacking histidine, which allows for selective germination of *MAT***a** meiotic progeny 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. Step 5, the *MAT***a** 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 *MAT***a** meiotic progeny are transferred to medium that contains both clonNAT and G418, which then selects for growth of double mutant (*bni1Δ::natR xxxΔ::kanR*). (See color plate section page xxx)

Because *can1Δ* is recessive it can be used as an additional haploid-selectable marker in the SGA procedure (see below) and we therefore often integrate the SGA reporters at the *CAN1* locus (Figure 2B). To facilitate a wide variety of genetic manipulations and improve

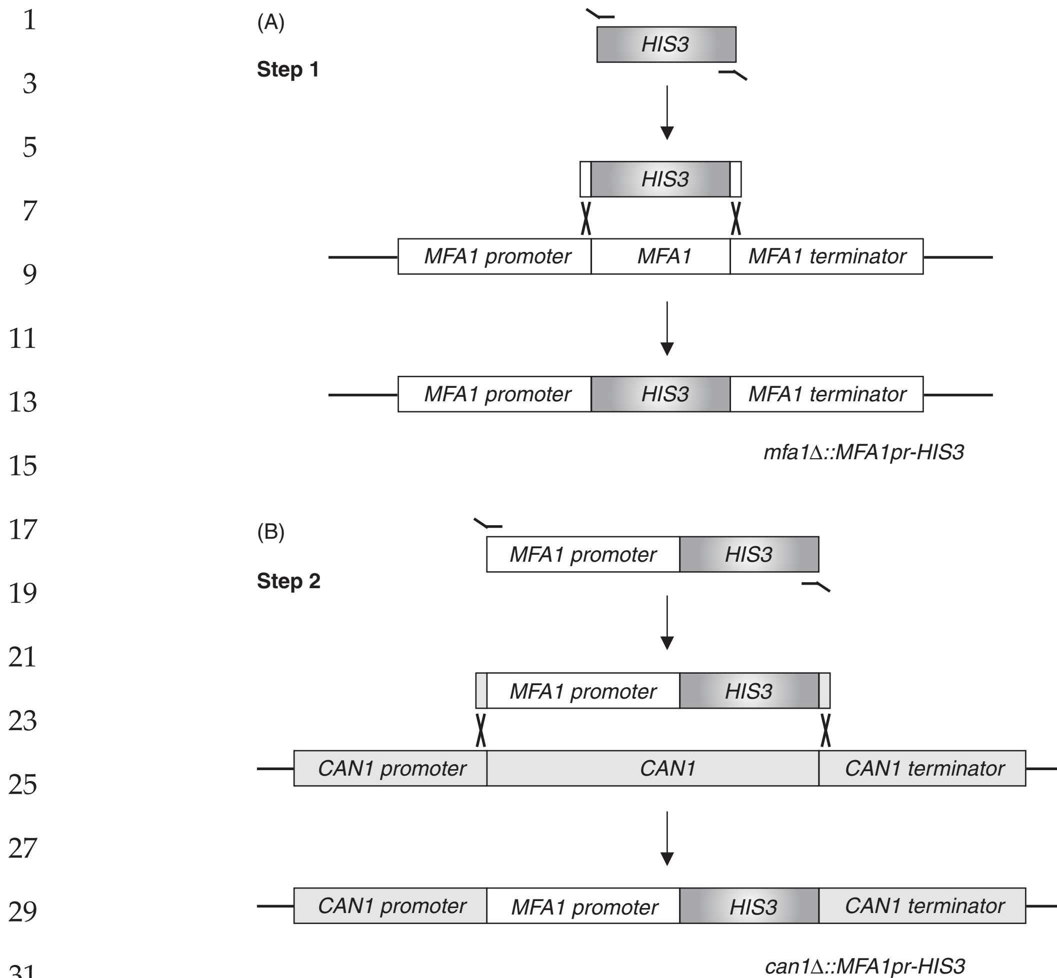

Figure 2. Construction of the SGA reporters. The construction of *can1Δ::MFA1pr-HIS3* involves two steps. (A) First, the *HIS3* open reading frame (ORF) is integrated at the *MFA1* locus, such that its expression is regulated by the *MFA1* promoter (*MFA1pr*), *mfa1Δ::MFA1pr-HIS3*. (B) Second, *MFA1pr-HIS3* is integrated at the *CAN1* locus, replacing the chromosomal copy of the *CAN1* gene, *can1Δ::MFA1pr-HIS3*.

the SGA selection, we also created a number of SGA reporters in which the **a**-specific promoter was fused to alternative selectable markers. In total, we utilized three selectable markers, the *S. cerevisiae LEU2* and *URA3* genes, as well as the *Schizosaccharomyces pombe his5* gene, which corresponds to the *S. cerevisiae HIS3* gene (see SGA reporter genotypes in Table 2). The Burke lab discovered that false negative SGA results may be derived from a gene conversion event in which a *HIS3*-based SGA reporter converts the *his3Δ1* deletion allele carried by the deletion mutant background to *HIS3* within the heterozygous diploids (see Figure 1, Step 2) (Daniel *et al*., 2005), thereby removing the mating-type specific selection for *MAT***a** meiotic progeny (see Figure 1, Step 4). This gene conversion

Table 1. List of a-specific genes

Gene	Description
MFA1	**a**-factor mating pheromone precursor
MFA2	**a**-factor mating pheromone precursor
STE2	α-factor receptor
STE6	**a**-factor exporter
BAR1	protease; cleaves and inactivates α-factor
ASG7	**a**-specific gene

Table 2. Yeast strains

Strain	Genotype	Source
Y2454	*MATα mfa1Δ::MFA1pr-HIS3 can1Δ ura3Δ0 leu2Δ0 his3Δ1 lys2Δ0*	Tong *et al.* (2001)
Y3068	*MATα can1Δ::MFA1pr-HIS3 ura3Δ0 leu2Δ0 his3Δ1 met15Δ0 lys2Δ0*	Tong *et al.* (2001)
Y3084	*MATα can1Δ::MFA1pr-HIS3 mfα1Δ::MFα1pr-LEU2 ura3Δ0 leu2Δ0 his3Δ1 met15Δ0 lys2Δ0*	Tong *et al.* (2004)
Y3656	*MATα can1Δ::MFA1pr-HIS3-MFα1pr-LEU2 ura3Δ0 leu2Δ0 his3Δ1 met15Δ0 lys2Δ0*	Tong *et al.* (2004)
Y5563	*MATα can1Δ::MFA1pr-HIS3 lyp1Δ ura3Δ0 leu2Δ0 his3Δ1 met15Δ0*	Tong *et al.* (2005)
Y5565	*MATα can1Δ::MFA1pr-HIS3 mfα1Δ::MFα1pr-LEU2 lyp1Δ ura3Δ0 leu2Δ0 his3Δ1 met15Δ0*	Tong *et al.* (2005)
Y6547	*MATα can1Δ::MFA1pr-LEU2 lyp1Δ ura3Δ0 leu2Δ0 his3Δ1 met15Δ0*	Boone Lab
Y7029	*MATα can1Δ::STE2pr-HIS3 lyp1Δ ura3Δ0 leu2Δ0 his3Δ1 met15Δ0*	Boone Lab
Y7033	*MATα can1Δ::MFA1pr-his5 lyp1Δ ura3Δ0 leu2Δ0 his3Δ1 met15Δ0*	Boone Lab
Y7039	*MATα can1Δ::STE2pr-LEU2 lyp1Δ ura3Δ0 leu2Δ0 his3Δ1 met15Δ0*	Boone Lab
Y7092	*MATα can1Δ::STE2pr-his5 lyp1Δ ura3Δ0 leu2Δ0 his3Δ1 met15Δ0*	Boone Lab
Y8205	*MATα can1Δ::STE2pr-his5 lyp1Δ::STE3pr-LEU2 ura3Δ0 leu2Δ0 his3Δ1 met15Δ0*	Boone Lab
Y8835	*MATα can1Δ::STE2pr-his5 lyp1Δ ura3Δ::natR leu2Δ0 his3Δ1 met15Δ0 cyh2*	Boone Lab
Y9230	*MATα can1Δ::STE2pr-URA3 lyp1Δ ura3Δ0 leu2,0 his3,1 met15,0*	Boone Lab

event is possible because the *his3Δ1* deletion only removes part of the *HIS3* open reading frame (Brachmann *et al.*, 1998). Since *S. pombe his5* does not share sequence similarity with *S. cerevisiae HIS3* there is no opportunity for gene conversion to occur. In the case of *LEU2* and *URA3*, the deletion mutant strain background carries a

1 complete deletion of the ORF, corresponding to the *leu2Δ0* and *ura3Δ0* alleles and therefore gene conversion is not an issue.

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(b) *can1Δ* and *lyp1Δ* markers

5 Because mitotic recombination can occur between homologous chromosomes in *MAT***a**/α diploids, a crossover event between the *MAT* locus and the centromere on chromosome III can result in *MAT***a**/**a** or *MAT*α/α diploids. In fact, streaking *MAT***a**/α diploid cells that carry the SGA reporter onto SD-His selects for *MAT***a**/**a** diploids. Because only a fraction (10%) of the heterozygous diploids (see Figure 1, Step 2) sporulate, rare mitotic crossover events can contribute to false negative scores, as a *MAT***a**/**a** diploid behaves like a *MAT***a** haploid, expressing *MFA1pr-HIS3*, and carries both deletion alleles. To avoid this complication, we introduced two recessive markers that confer drug resistance, *can1Δ* and *lyp1Δ*, into the query strain. The *CAN1* gene encodes an arginine permease that allows canavanine, a toxic analog for arginine, to enter and kill cells (Kitagawa, 1929; Sychrova and Chevallier, 1993). Similarly, the *LYP1* gene encodes a lysine permease that allows thialysine, a toxic analog for lysine, to enter and kill cells (Kitagawa, 1929; Sychrova and Chevallier, 1993). QA :1

Including *can1Δ* and *lyp1Δ* into the query strain means that *MAT***a**/**a** diploid cells are killed by canavanine and thialysine because they carry a wild-type copy of the *CAN1* and *LYP1* genes. Although it is possible for mitotic recombination to occur at the *can1Δ*, *lyp1Δ*, and *MAT* loci, it is unlikely for three independent recombination events (*MAT***a**/**a**, *can1Δ*/*can1Δ*, and *lyp1Δ*/*lyp1Δ*) to occur simultaneously within a cell. Hence, by introducing the *can1Δ* and *lyp1Δ* markers, the potential for *MAT***a**/**a** diploids to contribute to false negative SGA scores is reduced substantially.

(c) SGA starting strains

All strains are derivatives of BY4741 (*MAT***a** *ura3Δ0 leu2Δ0 his3Δ1 met15Δ0*) or BY4742 (*MAT*α *ura3Δ0 leu2Δ0 his3Δ1 lys2Δ0*) (Brachmann *et al*., 1998). Among the strains listed in Table 2, six, Y2454, Y3068, Y3084, Y3656, Y5563, and Y5565, were constructed previously and used for SGA analysis (Tong *et al*., 2001, 2004; Tong and Boone, 2005). Some of these strains, Y3084, Y3656, and Y5565, also carry an *MF*α*1pr-LEU2* reporter, which is activated only in *MAT*α cells, and enables selection of *MAT*α meiotic progeny during SGA analysis. The selection of *MAT*α meiotic progeny is also useful during the construction of *MAT*α SGA query strains by marker replacement of the original deletion mutant alleles, a method that avoids the construction of new alleles and has been outlined in detail previously (Tong and Boone, 2005).

Another seven strains, Y6547, Y7029, Y7033, Y7039, Y7092, Y8205, Y8835, and Y9230 (Table 2), are more recent developments; this set includes strains carrying the **a**-specific SGA reporter based on the

STE2 promoter and a variety of different selectable markers as discussed above. Y7092 (*MATα can1Δ::STE2pr-his5 lyp1Δ ura3Δ0 leu2Δ0 his3Δ1 met15Δ0*) is the starting strain we currently use for the construction of SGA query strains. With most of these starting strains, standard protocols for PCR-mediated integration or gene disruption are used to create SGA query strains; however, Y8205 also carries *STE3pr-LEU2* reporter, which is activated only in *MATα* cells and enables selection of *MATα* meiotic progeny and the construction of SGA starting strains by marker replacement of the original deletion mutant alleles (see Protocol 1).

(d) Media

Media used in the SGA analysis were described previously (Tong and Boone, 2005). Stock solutions are filtered-sterilized and stored in aliquots at 4°C: canavanine (50 mg/ml, Sigma); thialysine (50 mg/ml, Sigma); clonNAT (100 mg/ml, Werner Bioagents); and G418 (200 mg/ml, Invitrogen Life Technologies), and added to autoclaved medium. Solid medium contains 2% agar.

To minimize contamination on the deletion mutant array (DMA), we propagate it on YEPD+G418 medium. The query strain is mated to the DMA on YEPD. Diploids are selected on YEPD supplemented with 100 mg/l clonNAT and 200 mg/l G418. For efficient sporulation of diploids, the medium is supplemented with an amino-acid powder mixture (20 g/l agar, 10 g/l potassium acetate, 1 g/l yeast extract, 0.5 g/l glucose, 0.1 g/l amino-acids supplement). The amino-acids supplement for sporulation medium contains 2 g histidine, 10 g leucine, 2 g lysine, and 2 g uracil. Because ammonium sulfate impedes the function of G418 and clonNAT, synthetic medium containing these antibiotics are made with monosodium glutamic acid (MSG) as a nitrogen source. For selection of *MAT***a** meiotic progeny carrying *kanR* and, or *natR* markers, (SD/MSG) – His/Arg/Lys+canavanine/thialysine/G418, (SD/MSG) – His/Arg/Lys+canavanine/thialysine/clonNAT, (SD/MSG) – His/Arg/Lys+canavanine/thialysine/G418/clonNAT, the medium lacks histidine (selects for expression of *STE2pr-his5*), arginine, and lysine, and contains 50 mg/l canavanine (selects for *can1Δ*), 50 mg/l thialysine (selects for *lyp1Δ*), and 200 mg/l G418 (selects for *kanR*) and, or 100 mg/l clonNAT (selects for *natR*) [20 g/l agar, 20 g/l glucose, 1.7 g/l yeast nitrogen base w/o ammonium sulfate and amino acids (BD Difco), 1 g/l monosodium glutamic acid (Sigma), 2 g/l amino-acids supplement powder (DO – His/Arg/Lys)]. Tetrad analysis is performed on synthetic dextrose (SD/MSG) complete medium.

Protocol 1. SGA Procedure.

1. Set up cultures for query strain and the deletion mutant array (DMA) as follows:
   (i) Grow the query strain in a 5 ml overnight culture in YEPD.
   (ii) Replicate the 768-density DMA to fresh YEPD+G418. Let cells grow at 30°C for 2 days.
2. Pour the query strain culture over a YEPD plate, use the replicator to transfer liquid culture onto two fresh YEPD plates, generating a source of newly grown query cells for mating to the DMA in the density of 768.[1] Let cells grow at 30°C for 1 day.
3. Mate the query strain with the DMA by first pinning the 768-format query strain onto a fresh YEPD plate, and then pinning the DMA on top of the query cells.[2] Incubate the mating plates at room temperature for 1 day.
4. Pin the resulting *MAT***a**/α zygotes onto YEPD+G418/ clonNAT plates. Incubate the diploid-selection plates at 30°C for 2 days.
5. Pin diploid cells to enriched sporulation medium. Incubate the sporulation plates at 22°C for 5 days.[3]
6. Pin spores onto SD – His/Arg/Lys+canavanine/thialysine plates to select for *MAT***a** haploid meiotic progeny. Incubate the haploid-selection plates at 30°C for 2 days.
7. Pin the *MAT***a** meiotic progeny onto SD – His/Arg/Lys+canavanine/thialysine plates for a second round of haploid selection. Incubate the plates at 30°C for 1 day.
8. Pin the *MAT***a** meiotic progeny onto (SD/MSG) – His/Arg/ Lys+canavanine/thialysine/G418 plates to select for *MAT***a** meiotic progeny carrying the *kanR* marker. Incubate the *kanR*-selection plates at 30°C for 2 days.
9. Pin the *MAT***a** meiotic progeny onto (SD/MSG) – His/Arg/ Lys+canavanine/thialysine/G418/clonNAT plates to select for *MAT***a** meiotic progeny carrying both *kanR* and *natR* markers. Incubate the *kanR*/*natR*-selection plates at 30°C for 2 days.
10. Score double mutants for fitness defects.

2. Yeast cell manipulation

(a) Manual pin tools

An SGA screen can be performed manually using a 96 or 384 floating pin E-clip style manual replicator and registration tools such as a Colony Copier™, or Library Copier™. Hand-held replicator and accessories can be purchased from V & P Scientific, Inc (http://www.vp-scientific.com/floating_e-clip_replicators.htm).

To sterilize the replicator before and between each pinning step, the replicator is first placed in a tray of sterile water for ~1 min, which removes most of the yeast cells from the pins. Next, the replicator is placed in a tray of 10% bleach for 20 s, followed by three sequential rinses in different water baths (5 s/bath). Finally, the replicator is placed in 95% ethanol for 5 s. When excess ethanol drips off the pins, the replicator is flamed and allowed to cool before use.

To ensure the pins are cleaned properly and 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. 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 procedures in rotation.

(b) Robotic pin tools

There are a number of robotic systems available that can be programmed to manipulate yeast cell arrays such as: the VersArray colony arrayer system (BioRad Laboratories, http://www.bio-rad.com); the QBot, QPixXT, MegaPix (Genetix, http://www.genetix.co.uk); and the Singer Rotor HDA bench top robot (Singer Instruments, http://www.singerinst.co.uk).

The Rotor uses disposable plastic replicator pads, whereas most other machines use metal pinning tools, which must be sterilized between each pinning step. Because each robotic system has a different set up for the wash station, the following sterilization procedure is a general outline based on the VersArray colony arrayer system. To clean and sterilize the replicator prior to starting on the robot, the replicator is first placed in the sonicator that is filled with sterile water for 5 min. Next, the sonicator is cleaned and filled with 70% ethanol. The replicator is then placed in the sonicator for 5 min. Finally, the replicator is placed in 95% ethanol for 30 s and allowed to dry over the fan for 30 s.

To sterilize the replicator between each pinning step, the replicator is first placed in a tray of sterile water for 1 min to remove the cells on the pins. Next, the replicator is placed in a second tray of sterile water for 1 min. The replicator is then placed in the sonicator that is filled with 70% ethanol for 2 min. Finally, the replicator is placed in 95% ethanol for 30 s and allowed to dry over the fan for 30 s.

3. Array design

The collection of yeast deletion strains can be purchased from Invitrogen (http://www.resgen.com/products/YEASTD.php3);

American Type Culture Collection (http://www.atcc.org/common/specialCollections/cydac.cfm); EUROSCARF (http://www.uni-frankfurt.de/fb15/mikro/euroscarf/index.html); and Open Biosystems (http://www.openbiosystems.com/yeast_collections.php) as stamped 96-well agar plates or frozen stocks in 96-well plates.

The following procedure facilitates the transfer of yeast deletion strains from 96-well frozen stocks to solid agar medium and the building of high-density deletion mutant array (DMA). First, peel off the foil coverings slowly on the frozen 96-well microtiter plates. Second, allow the plates to thaw completely on a flat surface, preferably in a biological safety cabinet. Third, mix the glycerol stocks gently by stirring with a 96-pin hand-held replicator. Fourth, replicate the glycerol stocks from the 96-well plates onto YEPD+G418 agar plates. Take extreme caution that the pins do not drip liquid into neighboring wells. Finally, reseal the 96-well plates with fresh aluminum sealing tape, and return to -80°C. Allow cells to grow at room temperature for $\sim$2 days.

Because fitness is monitored as the output readout in SGA analysis, factors affecting the growth rate of yeast colonies can influence the system sensitivity. Yeast colonies grow faster and become larger in size when they have access to more nutrients in the medium. Hence, colonies surrounding an empty spot or those positioned along the edges of a high-density array, tend to be larger than the ones positioned in a dense area away from the edges (Figure 3A, see Colour Plate section). To minimize the positional effects and ensure a uniform growth rate in a high-density array, four important points need to be considered. First, slow-growing strains can be examined in a less biased manner by removing them from the regular array and creating a special one containing mutants with a slow growth rate. Second, a border can be added around the edges of the plate, i.e. the outermost layer of colonies on four edges of the plate, using a neutral strain carrying all the markers required in the experimental procedure. For example, the *MAT***a** *his3Δ::kanR* deletion strain for SGA analysis. Third, gaps or empty spots can be filled in or removed to make the array more robust for examining subtle differences in fitness amongst the deletion mutants. Fourth, each plate may contain a number of auxotrophic mutants which can be used for plate identification by providing a unique growth pattern or ''signature'' on medium lacking a specific nutrient (Figure 3B, see Colour Plate section).

A 384-density DMA can be assembled by spotting the strains manually or automatically using a colony arrayer. The collection of 384-density DMA plates can then be maintained as the master plate set for SGA analysis and also as frozen stock at -80°C. The agar plates can be kept at 4°C and propagated as needed, or revived from the frozen stock once every month. The 384-density array is also used as a source to generate working copies of the DMA in density formats such as 768 or 1536.

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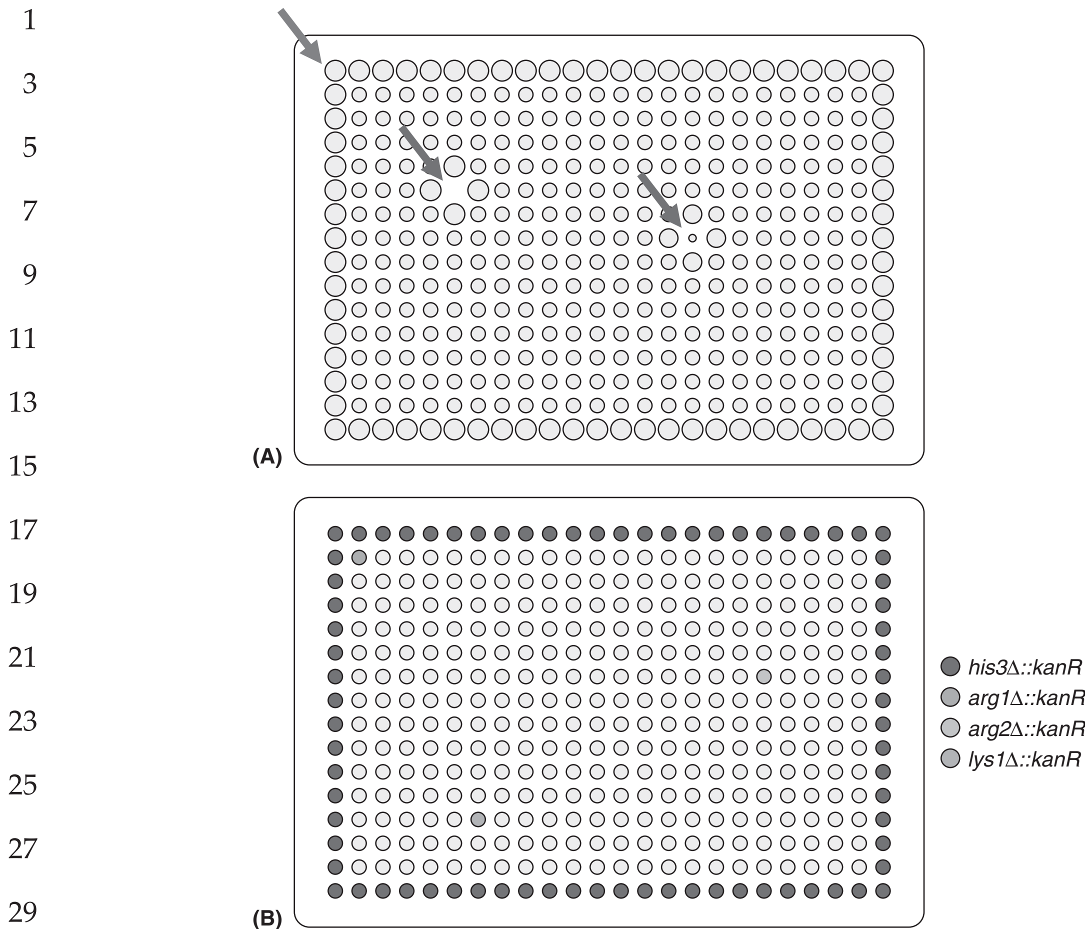

Figure 3. Array Design. Each spot represents a yeast colony growing in a 384-density array. (A) Yeast colonies surrounding an empty spot or a slow-growing strain (red arrows), and those positioned along the edges of the array (blue arrow), have access to more nutrients in the medium and therefore, tend to be larger than the ones positioned in a dense area away from the edges. (B) An ideal array layout for SGA analysis should facilitate accurate output readout and include the following: (i) removal of slow-growing strains from the regular array to a special array containing only mutants with a slow growth rate; (ii) a border around the edges of the plate, i.e. the outermost layer of colonies on four edges of the plate, using a neutral strain carrying all the markers required in the experimental procedure, for example the *MAT***a** *his3Δ::kanR* deletion strain (red colonies); (iii) filled in gaps or empty spots to make the array more robust for examining subtle differences in fitness amongst the deletion mutants; (iv) a number of auxotrophic mutants to be used as a unique plate identification system, for example, the *MAT***a** *ura4Δ::kanR* deletion strain (green colony), the *MAT***a** *trp1Δ::kanR* deletion strain (blue colony), and the *MAT***a** *lys1Δ::kanR* deletion strain (purple colony), are unable to grow on medium lacking uracil, tryptophan, and lysine, respectively. (See color plate section page xxx)

4. Scoring of putative interactions in an SGA screen

To evaluate the colony sizes of double-mutants generated from a query screen, we compare them to a reference set of wild-type

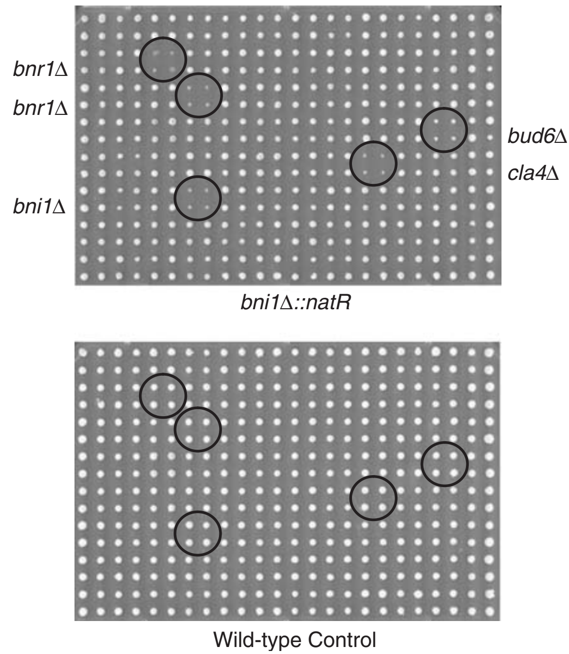

Figure 4. Examples of scoring synthetic lethal/sick interactions in an SGA screen. A *bni1Δ::natR* query strain is crossed to a test array containing 96 deletion mutants, each arrayed in quadruplicate in a square pattern. (note: SGA screens can be carried out at a density of 96, 384, 768 or 1536) *bnr1Δ* is duplicated within the array. The final array that selects for growth of the *bni1Δ* double mutants is shown at the top of the figure. Synthetic lethal/sick interactions lead to the formation of residual colonies (circled) that are smaller than the equivalent colony on the wild-type control plate. Synthetic lethal/sick interactions are scored with *bnr1Δ*, *cla4Δ*, and *bud6Δ*. When the query mutation is identical to one of the gene deletions within the array, double mutants cannot form because haploids carry a single copy of each allele; therefore, *bni1Δ* appeared synthetic lethal with itself.

control screens. The control set is generated by crossing *MATα ura3Δ::natR can1Δ::STE2pr-his5 lyp1Δ* to the DMA to create an output array carrying the SGA markers in every single-deletion mutant background. The double-deletion mutant array can be examined visually and compared to that of the wild-type control array. A synthetic lethal/sick interaction is scored when the colony size on the double-deletion mutant array is smaller than that on the wild-type control array (Figure 4). The query mutant is screened two more times, for a total of three independent screens. Screens can be carried out in 96, 384, 768 and 1536 density format with between 2 and 4 replica copies of each deletion mutant on the array.

In general, potential positive hits from three rounds of screening are combined and used to generate an unbiased set of putative interactions, which includes all those that appear two or three times in the three rounds of screening. A biased set of putative interactions is generated 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. 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. Both sets of putative interactions are then combined to create a list of candidates for confirmation.

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 (Tong *et al*., 2004). Following normalization of the images derived from control and double mutant plates, statistical significance can be determined for each strain by comparing the measurements between the mutants and wild-type controls.

5. Confirmation of the putative interactions generated from SGA analysis

To confirm the results obtained from SGA analysis, spores saved from the sporulation step in the SGA procedure (Figure 1, Step 3) can be used. Alternatively, heterozygous diploids of the query mutation and test mutation can also be generated independently by mating the *MATα* query strain to the *MAT***a** deletion strain of interest (*xxxΔ::kanR*). The resulting diploids can then be induced for sporulation and used in the RSA and tetrad analysis.

(a) Random spore analysis (RSA)

The following procedure facilitates RSA. First, inoculate a small amount of spores (approximately the size of a pinprick) in 1 ml of sterile water, and mix well. Second, plate 20 μl of suspended spores on SD – His/Arg/Lys+canavanine/thialysine medium, 40 μl of suspended spores on (SD/MSG) – His/Arg/Lys+canavanine/thialysine/G418, and (SD/MSG) – His/Arg/Lys+canavanine/thialysine/clonNAT, respectively, and 80 μl of suspended spores on (SD/MSG) – His/Arg/Lys+canavanine/thialysine/G418/clonNAT. Third, incubate the plates at 30°C for ~1.5–2 days. Finally, score the double-drug selection against the single-drug selections (Figure 5).

The expected number of *MAT***a** meiotic progeny on each medium should be roughly equal. SD – His/Arg/Lys+canavanine/thialysine allows germination of the *MAT***a** meiotic progeny that carries the *can1Δ::STE2pr-his5* and *lyp1Δ* markers. (SD/MSG) – His/Arg/Lys+canavanine/thialysine/G418 allows the germination of the *MAT***a** meiotic progeny that carries the *can1Δ::STE2pr-his5* and

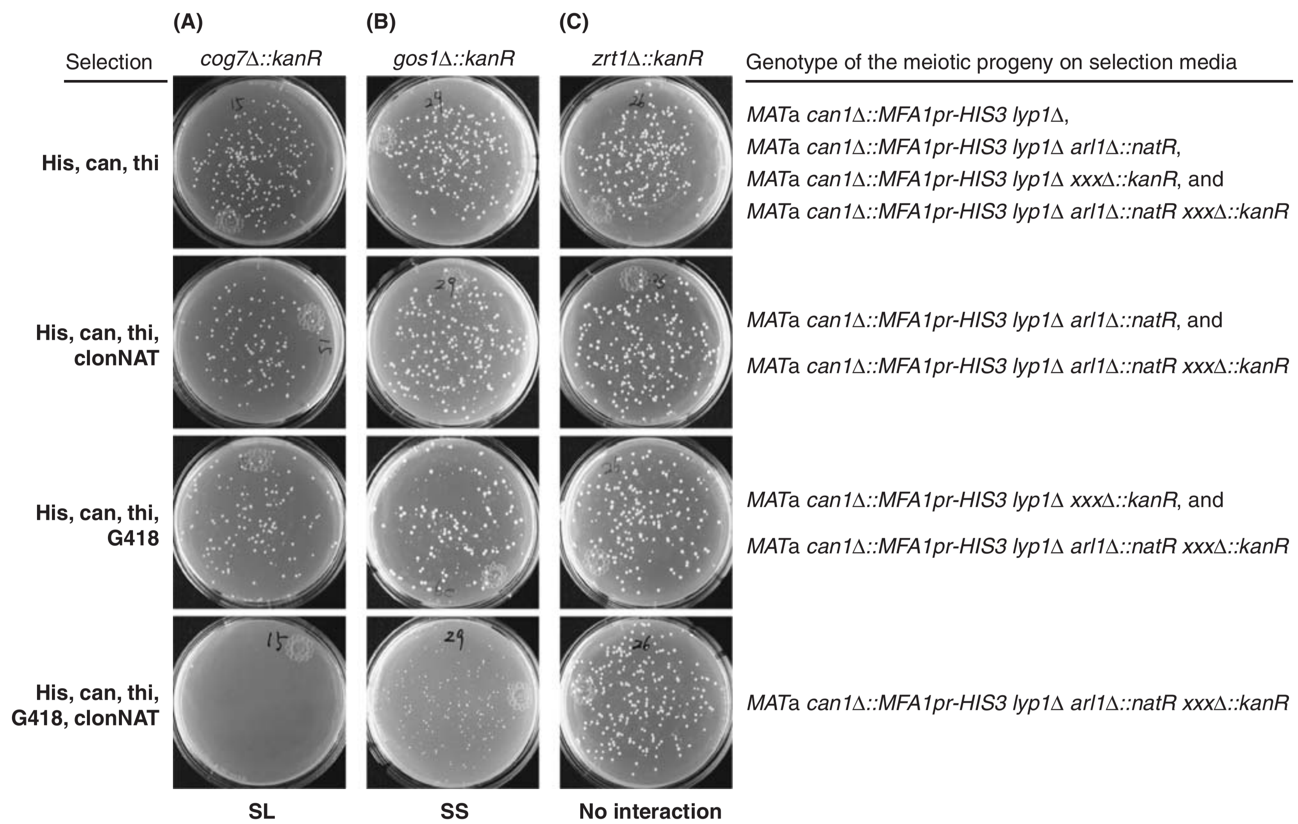

Figure 5. Examples of the random spore analysis (RSA): *MAT***a** meiotic progeny derived from sporulation of heterozygous diploids; *MAT***a**/α *arl1Δ::natR*/+ *cog7Δ::kanR*/+ (A), *MAT***a**/α *arl1Δ::natR*/+ *gos1Δ::kanR*/+ (B), and *MAT***a**/α *arl1Δ::natR*/+ *zrt1Δ::kanR*/+ (C), were plated onto media [SD – His/Arg/Lys+canavanine/thialysine], [(SD/MSG) – His/Arg/Lys+canavanine/thialysine/G418], [(SD/MSG) – His/Arg/Lys+canavanine/thialysine/clonNAT], and [(SD/MSG) – His/Arg/Lys+canavanine/thialysine/G418/clonNAT] as indicated. The plates were incubated at 30°C for ~2 days. Cell growth under the four conditions was compared and scored. The *MAT***a** *arl1Δ::natR cog7Δ::kanR* double mutant (A) was scored as having a synthetic lethal (SL) interaction. The *MAT***a** *arl1Δ::natR gos1Δ::kanR* double mutant (B) was scored as having a synthetic sick (SS) interaction. The *MAT***a** *arl1Δ::natR zrt1 Δ::kanR* double mutant (C) was scored as having no interaction.

lyp1Δ markers, and the *kanR*-marked gene deletion. (SD/MSG) – His/Arg/Lys+canavanine/thialysine/clonNAT allows the germination of the *MAT***a** meiotic progeny that carries the *can1Δ::STE2pr-his5* and *lyp1Δ* markers, and the *natR*-marked query deletion. (SD/MSG) – His/Arg/Lys+canavanine/thialysine/G418/clonNAT allows the germination of the *MAT***a** meiotic progeny that carries the *can1Δ::STE2pr-his5* and *lyp1Δ* markers, and the double mutations of the *natR*-marked query and *kanR*-marked gene deletion.

(b) Tetrad analysis

Standard procedure is followed to dissect tetrads except for the medium on which the spores are germinated. 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 resembles the final double mutant selection conditions (Figure 1, Step 6), only lacking G418 and clonNAT, and thus is more sensitive than the conventional rich medium in detecting subtle growth defects associated with the double mutant.

6. Applications of the SGA methodology

To examine synthetic genetic interactions with the essential genes, an SGA query strain can be crossed to an array of yeast mutants in which each essential gene has been placed under the control of the conditional Tetracycline-regulated promoter, the Tim Hughes Collection (yTHC) (Open Biosystems) (Figure 1), double mutants can be selected and scored for growth defects in the presence of doxycycline, which down-regulates the expression of the essential genes (Mnaimneh *et al*., 2004; Davierwala *et al*., 2005).

Because double mutants are created by meiotic recombination and since the viable gene deletion alleles represent mapping markers covering all chromosomes in the yeast genome, SGA screens also enable a genome-wide set of two-factor crosses that allow for high-resolution mapping of selectable traits, such as drug-resistant phenotypes or suppressors of temperature-sensitive mutations. In a proof-of-principle study, SGA mapping (SGAM) as applied to identify *ssd1Δ* as a suppressor of the lethality associated with deletion alleles of the RAM pathway (Jorgensen *et al*., 2002) and further application of SGAM identified *sgs1* mutations as suppressors of the slow growth defect associated with a *rmi1Δ* allele (Chang *et al*., 2005).

The SGA methodology is versatile because any genetic element (or any number of genetic elements) marked by a selectable marker(s) can be manipulated similarly. This array-based approach automates yeast genetics and can be easily adapted for a number of different screens, including higher order genetic interaction analysis (triple mutant genetic interactions) (Tong *et al*., 2004), dosage

lethality (Measday *et al*., 2005), suppression using high copy plasmid (dosage suppression), or plasmid shuffling. Mutant arrays generated by SGA can also be phenotypically assessed, for example, morphological analysis of genetic arrays using a high-throughput automated imaging system (Saito *et al*., 2004, 2005) will allow a detailed phenotypic assessment of double mutants. In addition, strain arrays generated by SGA can be used in secondary assays, for example, the *SCB::HIS3* reporter construct (Costanzo *et al*., 2004) was used to determine transcriptional responses in the ~5000 deletion mutant backgrounds. A yeast overexpression array, in which a wild-type strain was transformed with ~6000 different plasmids, each of which enables the conditional overexpression of a specific gene from the *GAL1* promoter (Zhu *et al*., 2001), has been assembled and can be used to screen for synthetic dosage lethality and suppression with SGA methodology (Sopko et al., unpublished data). QA :2 Other collections of yeast strains such as the green fluorescence protein (GFP) and tag-affinity protein (TAP) fusion libraries can also be integrated with the SGA methodology, allowing systematic examination of protein localization or the assembly of protein complexes in any genetic background.

◆◆◆◆◆◆ UNCITED REFERENCES

Kitagawa and Tomiyama, 1929.

◆◆◆◆◆◆ NOTES

1. Pinning the query strain and wild-type strain in the 768-format on agar plates is advantageous as cells are evenly transferred to the subsequent mating step.
2. One query plate should contain a sufficient amount of cells for mating with 6–8 plates of the DMA. The DMA can be reused for three to four rounds of mating reactions.
3. It is important to keep the sporulation plates at ~22–24°C for efficient sporulation. The resultant sporulation plates can be stored at 4°C for up to 4 months without significant loss of spore viability, and provide a source of spores for random spore analysis (RSA) and tetrad analysis.

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