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Quantitative genetic analysis in *Saccharomyces cerevisiae* using epistatic miniarray profiles (E-MAPs) and its application to chromatin functions

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Abstract

The use of the budding yeast *Saccharomyces cerevisiae* as a simple eukaryotic model system for the study of chromatin assembly and regulation has allowed rapid discovery of genes that influence this complex process. The functions of many of the proteins encoded by these genes have not yet been fully characterized. Here, we describe a high-throughput methodology that can be used to illuminate gene function and discuss its application to a set of genes involved in the creation, maintenance and remodeling of chromatin structure. Our technique, termed E-MAPs, involves the generation of quantitative genetic interaction maps that reveal the function and organization of cellular proteins and networks.

Keywords: Chromatin; Quantitative; Genetic interactions; Epistasis; E-MAP; *Saccharomyces cerevisiae*; DAmP

1. Introduction

The creation, maintenance and remodeling of chromatin structure occur through highly complex and regulated processes involving many transient protein interactions and modifications. These associations can be difficult to detect using standard techniques, thus necessitating the development of additional methods to study the relevant signal transduction pathways and their consequences. Genetic interactions, or the extent to which mutations in one gene modulate the phenotype of a second mutation, provide a functional view of cellular processes that is complementary to the picture provided by physical interactions [1,2]. In order to systematically collect information on these functional relationships, high-throughput technologies in *Saccharomyces cerevisiae* have been developed to qualitatively identify synthetic sick/lethal (SSL) (aggravating) genetic interactions between gene pairs on a genome-wide scale [3–5]. While these methods have proven very powerful, it has become clear that individual SSL relationships between non-essential genes are often hard to interpret since they usually identify genes that function in different, potentially parallel pathways [6–8]. Quantitative information on the entire spectrum of genetic interactions would provide a more comprehensive view of the cellular effects of gene mutation. This includes interactions that are alleviating (buffering or suppressing), where the double mutants grow more rapidly than would be expected given the growth rate of each of the single mutants. Such interactions often occur between pairs of genes working in the same cellular pathway [7–9]. To enable high-throughput analysis of the entire spectrum of interactions for large groups of genes we developed the E-MAPs (epistatic miniarray profiles) [7] technology that allows for the collection of quantitative genetic interaction data on logically selected subsets of genes.

To study chromatin functions, an E-MAP was generated that includes genes associated with chromatin functions, as well as those involved in transcription, DNA repair/recombination, DNA replication, chromosome segregation, and telomere function (Collins et al., submitted for publication).

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Several important predictions arising from this E-MAP for novel gene functions have recently been studied and confirmed [10–17]. One such prediction was a connection between *SET2*, a histone methyltransferase, and Rpd3C(S), a histone deacetylation complex that contains the chromodomain protein Eaf3p as well as Rco1p. E-MAP analysis of their genetic interactions showed a striking correlation between each of their profiles and that of *SET2* (Fig. 1A). Consistent with this result, the Δ*set2*, Δ*eaf3*, and Δ*rco1* strains all display alleviating genetic interactions with each other (Fig. 1B), suggesting that they function in the same cellular pathway. Indeed, methylation of histone H3 by Set2p has now been shown to be required for the recruitment and subsequent deacetylation activity of the small, Eaf3p and Rco1p containing Rpd3 complex to suppress spurious transcription initiation inside the coding regions of genes [11,18,19]. This example highlights the power of genetic interactions for revealing functional dependencies that could not have been observed by protein–protein interaction data alone.

An important aspect of E-MAPs is their quantitative scoring system that identifies both aggravating and alleviating interactions. This ability is dependent on the presence of a high density of interactions [7,20], which improves the signal-to-noise ratio relative to full genome screens. Indeed, screening a single query gene against the whole-genome may actually result in the detection of fewer genetic interactions than screening against a smaller set of functionally related genes despite the larger number of potential interactors. This point can be illustrated by observing the drastic decrease in the confidence assigned to each interaction (as a function of interaction score) when a whole-genome screen situation is simulated (tenfold increase in the number of non-interacting gene pairs) (Fig. 2). Hence, it is of great value to select small, comprehensive, subsets of functionally related genes for quantitative E-MAP analysis. Additionally, it is now feasible to include all relevant essential genes in the analysis by employing DAmP (decreased abundance by mRNA perturbation) technology [7].

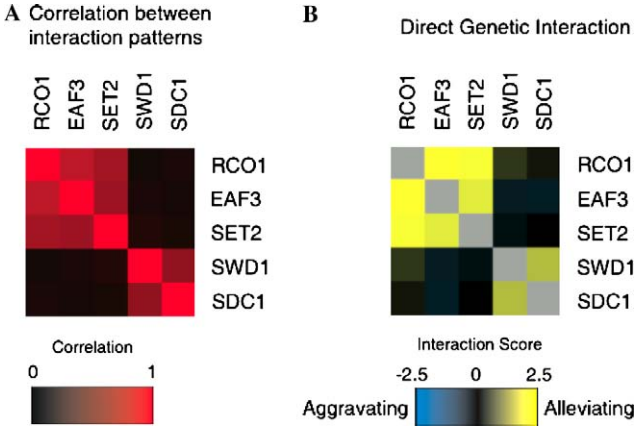

Fig. 1. (A) The colorscale represents the correlation between the patterns of genetic interactions measured in E-MAP experiments for the genes encoding Rpd3C(S) complex members *RCO1* and *EAF3*, and the histone methyltransferase *SET2*. Notice that the correlation is specific to *SET2* as the genes encoding members of the Set1p-containing complex *SWD1* and *SDC1* do not exhibit such correlation. (B) A map of the direct genetic interactions observed between the same genes shown in (A).

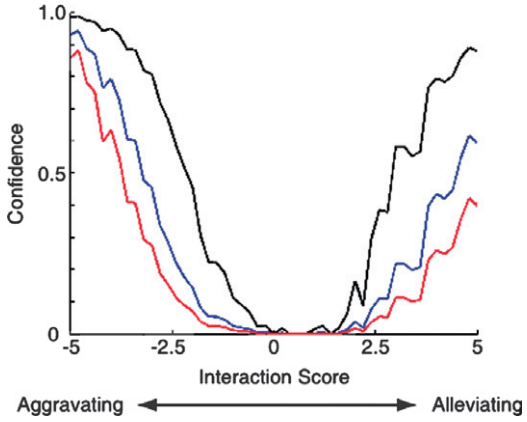

Fig. 2. Shown are plots of an estimate of the fraction of gene pairs with the indicated interaction score that correspond to genuine interactions. The curves correspond to data from an early secretory pathway E-MAP [7] (black) and the same data with a simulated fourfold (blue) or tenfold (red) increase the number of non-interacting gene pairs. The curves were computed as previously described [20].

Here we discuss the basic approach for generating an E-MAP. Specifically, we review the considerations in choosing a set of genes for analysis by E-MAP, the strain construction procedures for creating both gene deletions and DAmP alleles, and the manual pinning protocols that allow the generation of all double mutant combinations. Many of these protocols rely on previously published synthetic genetic array (SGA) protocols [21] and can also be found on our website (http://www.weissmanlab.ucsf.edu). The analytical tools and software (which is freely distributed), necessary for computing quantitative genetic interaction scores once colonies of double mutant strains have been obtained, have been described in detail elsewhere [20].

2. Selection of genes for E-MAP analysis

Previous efforts to gather genome-wide genetic interaction data [3,5] have demonstrated that most genes only show strong aggravating interactions with a relatively small percentage of the genome, and that these interactions tend to be with functionally related genes. Based on these considerations, E-MAPs were developed to measure all genetic interactions between 384 functionally related mutations. This number is compatible with commercially available hand-pinners, which can be used for rapid and relatively inexpensive manual generation of yeast libraries containing all pair-wise combinations of mutations (approximately 150,000 double mutant strains). By focusing on functionally related genes, an interaction map that contains a large fraction of all interactions for each of the genes is generated [7,20].

Thus, the success of the E-MAP approach depends on a wise selection of a gene subset, which should include

both well-studied proteins, serving as controls, alongside uncharacterized genes for which this method can provide new functional predictions. The best strategy for selecting this subset depends on the biological process being studied. For example, sub-cellular localization of the entire yeast proteome [22] allows the definition of gene sets based on the localization of their protein products to specific sub-cellular compartments (e.g., mitochondria, nucleolus, endosomes). Indeed, an E-MAP created largely on the basis of protein sub-cellular localization to the early secretory pathway (endoplasmic reticulum, Golgi apparatus, and the cycling vesicles) yielded a large quantity of biologically relevant data [7]. Selection of gene subset could also be based on groups of enzymes and substrates involved in particular modifications (e.g., ubiquitination, sumoylation, and phosphorylation) or general cellular processes (e.g., meiosis, cell cycle, and stress response) where proteins do not necessarily localize to the same cellular compartment but are nevertheless functionally related. Comprehensive physical interaction datasets [23–25], are an additional tool for defining gene groups. Such information on the large majority of complexes in the cell, could be used to create an E-MAP in which many cellular functions are represented thus becoming a platform whereby uncharacterized proteins could be genetically implicated in one or more biological processes. Finally, a "boot-strapping" approach can be used where selection of genes can be based on a limited number of whole genome genetic screens complemented with genes that show similar patterns of gene expression, physical interactions or genetic interactions. The E-MAP focusing on chromatin function that we have recently generated (Collins et al., submitted for publication) is an example of the large number of ways in which a functionally coherent gene subset can be chosen based on general criteria including protein–protein interaction data, qualitative genome-wide SSL screens, gene expression data, and manual curation of the literature.

3. Generation of mutant strains for E-MAPs

3.1. Deletion strains

Once the set of non-essential genes to analyze has been chosen, it is necessary to generate complementary MATa and MATα deletion strains. The MATa set can be obtained from the commercially available yeast deletion consortium library (*his3*Δ1 *leu2*Δ0 *met15*Δ0 *ura3*Δ0) that contains individual non-essential genes deleted with a kanamycin resistance (Kan^r) cassette [26]. However, these strains lack any of the markers necessary for performing the mating and spore selection steps in high-throughput [4,21]. Therefore, the MATα query strain library must harbor all of these genetic elements (*his3Δ1 leu2Δ0 LYS2+ met15Δ0 ura3Δ0 Δcan1::MATaPr-HIS3 Δlyp1::MATαPr-LEU2*). Critical to these strains are the two recessive alleles of *CAN1* and *LYP1* that can be used to select against heterozygous diploids (the presence of a functional *LYP1* or *CAN1* allele confers sensitivity to S-AEC (Sigma) or Canavanine (Sigma), respectively) and selectable markers for specific spore mating types (MATaPr-HIS3; MATαPr-LEU2) [21]. A starter strain containing these required elements is transformed in a high-throughput manner to create deletion strains using a selectable marker. The markers typically used are the *URA3* gene or the *NAT^r* gene (which confers resistance to Nourseothricin (NAT, Werner bioagents) and can be amplified from the pFA6-NAT-MX6 [27] plasmid). Typically, two rounds of high-throughput transformation generate 70–80% of the strains to be constructed. Additional individual strains can be made if required by repeating these protocols or by using traditional transformation procedures [28].

3.1.1. Generate PCR products for transformation

1. Make PCR mix.

per reaction:	*per 100 reactions*
25 μl TAQ PRO complete (Denville Scientific)	2.5 ml
1 μl of 100 ng/μl pFA6-NAT-MX6 Plasmid (templ. For NAT^r)	100 μl
14 μl DDW	1.4 ml

2. Mix well and distribute 40 μl into each well of a 96-well PCR plate.
3. Add 10 μl of 5 μM primer mix (Forward and Reverse) into each well and mix by pippeting.
4. Seal plate using PCR plate cover (Bellco Glass, Inc) and run PCR in a 96-well block using the following cycling parameters:
   1. 94 °C 5′
   2. 94 °C 30″
   3. 60 °C 30″
   4. 72 °C 1.5′
   5. go back to 2, 29 times
   6. 72 °C 10′
   7. 4 °C forever
   8. END

* No cleaning of the PCR product before transformation is necessary, although PCR products should be checked by agarose gel electrophoresis if transformation efficiency is low.

* The forward primers are designed such that they have homology regions to the 40 bp upstream of the start codon (including) of the gene to be deleted, followed by 20 bp of homology to the plasmid (F2, [29]). For the reverse primer, the 40 bp of homology are downstream of the stop codon and are followed by 20 bp of homology to the plasmid (R2, [29]).

3.1.2. Transform appropriate cells

1. Grow cells in YEPD so that they are at least 5–6 cell divisions into mid log phase following dilution from saturated culture and are at $OD_{600} \approx 0.7$ ($\sim 1.5 \times 10^7$ cfu/ml).
2. Pellet 3 ml ($\sim 4–5 \times 10^7$ cells) of culture/ transformation by centrifugation (2500RCF 5′) at room temperature (Notice: for a 96-well plate ($\sim$100 transformations) this will correspond to 300 ml of cultured cells). Discard supernatant.
3. Wash cells twice in 1/10 vol of 100 mM LiAc.
4. Re-suspend cells in 1/100 vol of 100 mM LiAc.
5. Prepare transformation mixture.

per reaction:	*per 100 reactions*
100 μl 50% (w/v) PEG 3350 (Filter sterilized)	10 ml
15 μl 1 M LiAc (Filter sterilized)	1.5 ml
20 μl 2 mg/ml carrier DNA (Boiled for 3′ and than put on ice)	2 ml
30 μl cell suspension	3 ml
18 μl Hybri-Max DMSO (Sigma)	1.8 ml

6. Mix well and distribute 183 μl into each well of a 96-well PCR plate.
7. Add 15 μl transforming DNA (PCR product) and mix by pippeting. Seal plate using PCR plate cover (Bellco Glass, Inc).
8. In a PCR block incubate at 30 °C for 30′, then 42 °C for 15′.
9. Pellet cells for 1′ at 2500RCF and discard supernatant. In 96-well PCR plates this can be done by rapidly shaking off excess liquid as the conal structure of the tube retains the cells.
10. Re-suspend in 50 μl YEPD.
11. If using an antibiotics resistance marker (i.e., Kan^r or NAT^r) recover for 3 h at 30 °C. Otherwise proceed directly to the next step.
12. Plate entire volume onto selective medium. Colonies will appear after 2 days.

* PEG should be prepared fresh before each transformation. Low efficiency of transformation is most often due to the PEG changing from the 50% w/v ratio [28].
* To enable the rapid plating of large numbers of colonies in 96-well format, it is possible to use two 48-well plates (Genetix) since they have wells that are large enough to resolve many colonies.

3.1.3. Check PCR for correct colonies

1. Pick transformed colony into 1 ml of selection media using 96-well 2 ml (deep-well) plates (VWR).
2. Grow cells to stationary phase ($\sim$24 h).
3. Transfer 200 μl of each culture to 96-well PCR plates.
4. Spin down cells (2500 RCF for 2′) and discard supernatant by aspiration or rapid shake.
5. Re-suspend in 50 μl of 20 mM NaOH + 0.1 mg/ml RNaseA and seal plate using PCR plate cover.
6. Boil cell suspension at 100 °C for 25′ (can use thermocycler).
7. Spin down cells (2500 RCF for 2′).
8. Use 2 μl of the supernatant per 20 μl PCR reaction.
9. Make PCR mix.

per reaction:	*per 100 reactions*
10 μl TAQ PRO complete	1 ml
4 μl DDW	400 μl
2 μl F2CHK primer (5 μM)	200 μl

F2CHK primer sequence: AACCCGGGGATCCGTCGACC [29].

10. Distribute 16 μl into each well in a 96-well PCR plate.
11. Add 2 μl of 5 μM gene specific forward check primer into each well and mix by pippeting.
12. Add 2 μl of boiled cell suspension into each well and mix by pippeting.
13. Seal plate using PCR plate cover and run PCR in a 96-well block using the same cycling parameters as above.
14. Visualize products following agarose gel electrophoresis.

* Forward check primers should anneal to sequences $\sim$300–500 bp upstream of the start codon to produce short, easily viewed PCR products.
* To enable rapid viewing of 96 PCR products in parallel, it is recommended to use ready-made 96-well gels (Invitrogen, E-Gel 96). These can be loaded using a liquid handling robot (such as a BIO-MEK) or manually using a multi channel pipette.

3.2. DAmP strains

A critical feature of the E-MAP is the high density of genetic interactions which is achieved both through the selection of informative groups of genes and through the ability to incorporate essential genes in the analysis. In the past, high-throughput generation of mutations in essential genes has been difficult. For example, creation of a suitable library of temperature-sensitive mutants is very laborious and the analysis must be carried out under non-permissive conditions, which may influence the type and severity of the genetic interactions observed. The use of TET-promoter shut-off alleles [30] has enabled more comprehensive use of essential genes in genetic interaction screens. However, some of these strains are not well suited for large-scale genetic interaction studies due to difficulties in achieving intermediate expression levels. A complementary method for systematically creating hypomorphs of essential genes is the DAmP (decreased abundance by mRNA perturbation) technique [7], which creates destabilized mRNA transcripts by disrupting the 3′UTR of an essential gene through integration of an antibiotic selectable marker immediately downstream of the stop codon. This approach allows for constitutively decreased expres-

sion of genes that are still regulated from their natural promoters. DAmP alleles typically maintain cell viability but tend to have genetic interaction patterns resembling those of non-essential genes in the same cellular pathway [7]. In addition, this method is amenable for drug screening as strains with lower expression levels of genes necessary for specific drug resistance may become more susceptible to treatment with the appropriate drug. DAmP alleles can be created in high-throughput using the following protocols:

3.2.1. Generate PCR products for transformation

As described above. The plasmids used as PCR templates for the selection markers are pFA6-KAN-MX6 [29] for the MATa strains and pFA6-NAT-MX6 [27] for the MATα strains. The forward primers are designed to contain homology to the 40 bp upstream to, and including the, stop codon (so that a stop codon is retained in the transgene) followed by 20 bp of homology to the plasmid (F2). For the reverse primer, the homology regions should be chosen to incorporate the 40 bp immediately downstream of the stop codon (such that no 3′UTR remains following integration), followed by 20 bp of homology to the plasmid (R2). This ensures that the 3′UTR is disrupted and replaced by a selection marker (Fig. 3).

3.2.2. Transform appropriate cells

The transformation protocol is the same as described above. However, it is recommended to transform diploid cells to avoid accumulation of suppressors during the transformation procedure. The transformed diploids can also be frozen down to allow easy regeneration of the haploid strains if one should become contaminated. For the MATα strains, a starter diploid strain that includes all markers necessary for SGA is used [21] and this facilitates the rapid creation of haploid strains through random spore analysis instead of manual dissection of tetrads. The recommended diploid genotype is: *his3Δ1/his3Δ1 leu2Δ0/leu2Δ0 LYS2+/LYS2+ met15Δ0/met15Δ0 ura3Δ0/ura3Δ0 can1Δ::MATaPr-HIS3/CAN1 lyp1Δ::MATαPr-LEU2/LYP1*.

To allow for high-throughput selection of MATa spores, we have created a plasmid carrying the *S. pombe HIS5* gene (which is homologous to *S. cerevisiae HIS3*) expressed from the MATa specific *STE2* promoter and present on pRS316 carrying a *URA3* selection marker. Diploids (*his3Δ1/hi3Δ1 leu2Δ0/leu2Δ0 LYS2+/LYS2+ met15Δ0/met15Δ0 ura3Δ0/ura3Δ0 CYH2/cyh2*) carrying the DAmP allele are transformed with this plasmid and the transformed diploids are sporulated, zymolased and plated on SD (With monosodium glutamate)-HIS+G418+cycloheximide (10 mg/L). This allows for selection of single MATa clones. Once haploids have been selected it is possible to counter-select against the plasmid by plating on 5-FOA thus restoring the original marker genotype of the MATa strains.

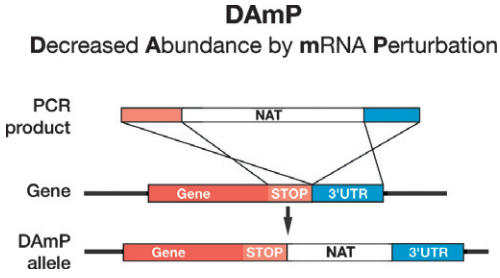

Fig. 3. Schematic representation of DAmP technology.

3.2.3. Check PCR for correct colonies

As described above. Check primers are designed to anneal to the sequences 300–500 bp upstream of the stop codon.

3.2.4. Sporulate to haploids

1. For correct clones, transfer 200 μl of saturated culture to a 96-well 2 ml (deep-well) plate.
2. Spin down cells and discard supernatant by aspiration.
3. Re-suspend cells in 200 μl of SPO media (Use SPO recipe below without the agar).
4. Incubate for 5 days shaking at 30 °C. It is critical to allow oxygenation of the media during this incubation period.

3.2.5. Select for correct haploids

1. Transfer 20 μl of sporulating cells into a 96-well PCR plate.
2. Spin down cells and discard supernatant.
3. Re-suspend cells in 20 μl of zymolase (0.5 μg/μl in 1 M sorbitol, 10 mM Tris pH 7.5).
4. Incubate at 30 °C for 45′.
5. Add 200 μl 1 M sorbitol to stop the reaction. (This solution containing the spores can be sealed and kept at 4 °C for up to a month).
6. Plate 50 μl cells on correct selection plate (it is possible to use 48-well plates as described above). To select against the diploids and for the MATα haploids in this suggested strain the medium used is: SD (with monosodium glutamate) +NAT +S-AEC +CAN–LEU–ARG –LYS.
7. Once haploids grow, repeat check PCR procedure for 2 clones from each strain and transfer correct final clone to the final growth plate for maintenance and freeze down.

4. Creation of double mutant strains

Once the strains have been made, the creation of double mutants can be performed in high-throughput using the previously published protocols [21]. However, to allow this method to be widely used, we have optimized our protocols to use only manual pinning tools and grids (V & P scientific, San Diego, CA). Most of the steps are performed using pin tools with large-diameter pins so that adequate amounts of cells are carried over from step to step (VP384F6 alongside library copiers for liquid VP381 and for agar VP380). The final pinning onto double mutant selection plates is performed using a pinner with small-diameter pins (VP384FP4 alongside library copier VP380N) to deposit smaller foci of cells thereby maximizing the measured growth differences between strains.

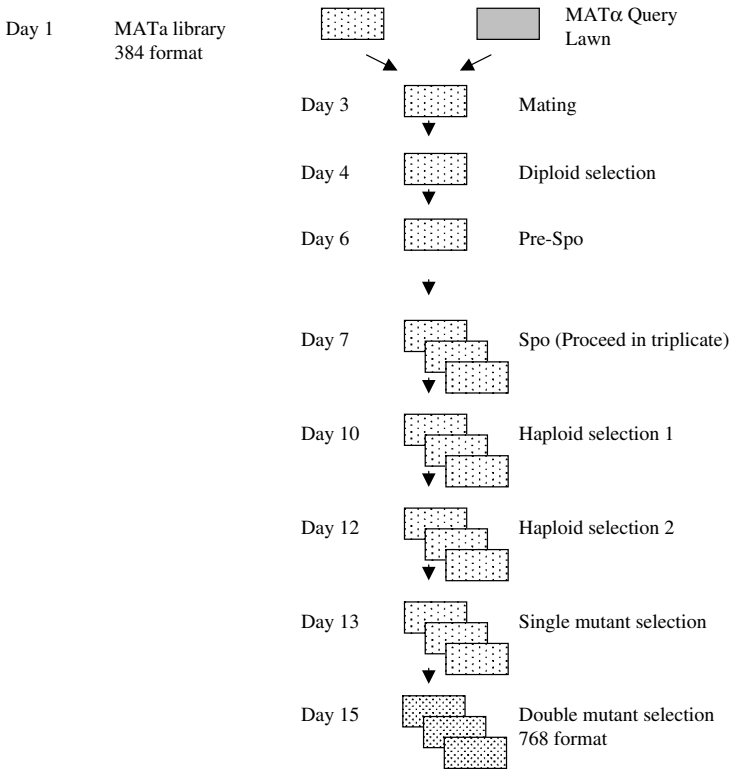

Fig. 4. Flow chart of pinning steps for creation of double mutant strains in high-throughput.

The pinning of multiple plates can be time consuming. By working with 3 pin tools in parallel it is possible to accelerate the procedure. Pin tools are subject to 5 wash steps before each use:

1. 30″ DDW (It is recommended to place a fine pore nitrocellulose sponge (VWR) in the bath and dip the pinner up and down three times to physically shear off yeast cells from the pin heads).
2. 30″ 10% Bleach (With sponge)
3. 30″ DDW
4. 30″ 100% EtOH
5. Dip in 100% EtOH followed by quick "torching" in flame of Bunsen Burner.

The entire protocol for creating double mutant strains can be followed using the following schedule (For flow chart see Fig. 4):

4.1. Day1—set up

- Plate fresh copy of the MATa strains in 384 format on YPAD + Kan omni-tray plates.
- Make lawn of MATα strain on YPAD + NAT omni-tray plates.
- Incubate plates for 2d at 30 °C.

* During all incubations plates should be covered to avoid drying.

4.2. Day3—mating

- Mate strains by pinning once from the lawn and once from the library onto a YPAD plate.
- Leave at RT for 1d.

4.3. Day4—diploid selection

- Replicate mating plate to diploid selection plate (YPAD + Kan + NAT).
- Incubate plates for 2d at 30 °C.

4.4. Day6—pre-SPO

- Replicate to pre-SPO.
- Incubate plates for 1d at 30 °C.

4.5. Day7—SPO

- Replicate to Spo plates. At this stage triplicate plates should be created and maintained from here on to allow accurate statistical analysis of the resulting growth phenotypes.
- Incubate plates for 5d at 22 °C.

* Cells should be pinned three separate times to insure maximal transfer of diploid cells to the SPO media.
* At this stage it is extremely important that plates are NOT covered (to allow for oxygen dependant synthesis

of new cell membrane phospholipids). In order to prevent drying they can be grown in a humidified incubator or with wet towels around them.

4.6. Day10—first haploid selection

- Replicate to first haploid selection (HS) plates (to select for MATa haploid spores).
- Incubate plates for 2d at 30 °C.

4.7. Day12—second haploid selection

- Replicate to second HS plates.
- Incubate plates for 1d at 30 °C.

4.8. Day13—single mutant selection

- Replicate to single mutant (SM) selection plates (to select for MATa haploid spores that are Kan^r).
- Incubate plates for 2d at 30 °C.

4.9. Day 15—double mutant selection

- Replicate to double mutant (DM) selection plates (to select for MATa haploid spores that are Kan^r and NAT^r).
- Incubate plates for 2d at 30 °C.

* At this stage each 384 plate is replicated into a 768 format in which an internal duplicate is pinned by using the diagonal of each plating position (Fig. 5) to improve measurement precision.

4.10. Day 17—analysis

- Photograph plates.

* pictures are taken with a digital camera (Canon Powershot G2, 4.0 Megapixels) that is mounted on a KAISER RS 1 camera stand (product code-no. 5510) with illumination from two Testrite 16X24 Light Boxes (Freestyle Photographic Supplies product #1624).

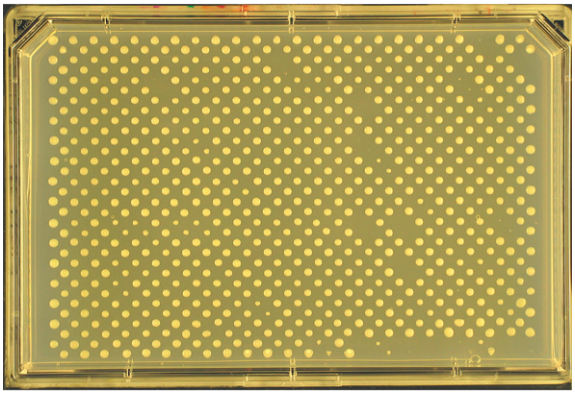

Fig. 5. Picture of a "Double Mutant" plate pinned in 768 colony format using a VP384FP4 pinner.

Once plates have been photographed they can be analyzed to produce quantitative E-MAP scores using our freely distributed software and methods (described in [20] and on our website (http://www.weissmanlab.ucsf.edu)).

5. Perspective

Although a large number of accurate predictions have already been generated using our E-MAP focused on chromatin-related functions, many additional discoveries of gene function have yet to be made using these data. The E-MAP approach, however, is general and allows the study of any group of functionally related genes. Due to the optimization of this protocol to fit manual pinning tools, it is now feasible for any yeast lab to create an E-MAP tailored to their genes/ processes of interest. The choice of smaller subsets of genes for analysis is also amenable for genetic studies in higher organisms where whole-genome screens are less feasible.

Media recipes (Based on [21])

Pinning procedures are performed in omni-tray plates (nunc)

All recipes are for 1 l = ~30 plates

All plates should be stored in the dark at 4 °C **YPAD (YEPD + ADE)**

1. Mix 120 mg Adenine + 10 g yeast extract + 20 g peptone + 20 g agar
2. Dislove in DDW to 950 ml
3. Autoclave
4. After autoclave add 50 ml of 40% glucose
5. Pour into plates

For **YPAD + NAT** Add 200 mg NAT after autoclave **YPAD + Kan** Add 200 mg G418 after autoclave **YPAD + NAT + Kan** Add 200 mg of NAT + G418 after autoclave **Pre-SPO**

1. Flask1: 10 g Difco yeast extract + 20 g Difco Agar + 500 ml DDW
2. Flask2: 30 g Difco nutrient broth + 375 ml DDW
3. Flask3: 50 g Dextrose + 125 ml DDW
4. Autoclave all three flasks
5. After autoclaving mix flasks and pour into plates

SPO

1. Flask1: 20 g Agar + 820 ml DDW
2. Flask2: 0.5 g amino acid—ura–trp powder mix, 2.5 ml 20 mM uracil stock, 2.5 ml 20 mM tryptophan stock, 163 ml DDW
3. Autoclave each flask separately
4. Add 20 ml of 500 mg/ml **filter sterilized** potassium acetate (do not autoclave the KoAc!) to flask 2.
5. Mix flask1 and flask2 and pour into plates

Haploid selection plates (HS)

1. Flask1: 20 g Agar + 850 ml DDW
2. Flask2: 6.7 g YNB w/o amino acids + 2 g "DROP OUT" Mix (recipe below) + 100 ml DDW
3. Autoclave
4. After autoclave mix flask 1 and flask 2
5. Add: 50 ml of 40% glucose + 0.5 ml of 100 mg/ml canavanine + 0.5 ml of 100 mg/ml S-AEC
6. Pour into plates

Single and double mutant selection plates (SM + DM)

1. Flask1: 20 g Agar + 850 ml DDW
2. Flask2: 1.7 g YNB w/o amino acids and w/o ammonium sulfate + 2 g "DROP OUT" Mix (recipe below) + 1 g Monosodium glutamic acid + 100 ml DDW
3. Filter sterilize flask2
4. Autoclave flask1
5. mix flask1 and flask2
6. Add: 50 ml of 40% glucose + 0.5 ml of 100 mg/ml canavanine + 0.5 ml of 100 mg/ml S-AEC
7. For Single mutant plates add 200 mg G418.

For Double mutant plates add 200 mg G418 + 200 mg NAT.

DROP OUT mix

Adenine	3 g
Alanine	2 g
Aspargine	2 g
Aspartic acid	2 g
Cysteine	2 g
Glutamine	2 g
Glutamic Acid	2 g
Glycine	2 g
Inositol	2 g
Isoleucine	2 g
Leucine	10 g
Methionine	2 g
p-Aminobenzoic acid	0.2 g
Phenylalanine	2 g
Proline	2 g
Serine	2 g
Threonine	2 g
Tryptophan	2 g
Tyrosine	2 g
Uracil	2 g
Valine	2 g

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