Chapter 4

Ultrahigh-Density Screens for Genome-Wide Yeast EMAPs in a Single Plate

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Abstract

Systematic measurements of genetic interactions have been used to classify gene functions and to categorize genes into protein complexes, functional pathways and biological processes. This protocol describes how to perform a high-throughput genetic interaction screen in *S. cerevisiae* using a variant of epistatic miniarray profiles (E-MAP) in which the fitnesses of 6144 colonies are measured simultaneously. We also describe the computational methods to analyze the resulting data.

Key words EMAP, Genetic interactions, Synthetic lethality, Ultrahigh-density yeast arrays, S-scores

1 Introduction

Genetic interactions are defined as the deviation in an observed phenotype of a double mutant from what would be expected given two independent genetic mutations [1]. Disrupting genes in the same pathway or biological process may have large functional consequences, which may not be reflected by physical binding or similar transcriptional regulation [2, 3]. As such, accurately quantifying genetic interactions is an essential component of reconstructing the complex networks that define cell function, aid in elucidating the underpinnings of disease, and highlight potential therapeutic targets. However, given the size of the human genome, systematically profiling all potential interactions between different isoforms combinations is untenable. The budding yeast, *Saccharomyces cerevisiae*, has proved to be a valuable model to quantitatively measure the growth defects of a large selection of double mutant strains before translating interesting findings to screens in mammalian cell lines [4–6].

To construct double mutant yeast libraries, individual knockouts are mated in a systematic and often automated fashion; a strain

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carrying a single gene knockout is mated to an ordered array of other single gene knockouts [2]. The use of large-scale array formats greatly decreases the time and cost associated with genetic interaction screens. These studies can provide a better contextual understanding of gene function beyond a single interaction measurement. By correlating pairwise genetic interaction profiles of query genes across all knockouts in the array, a measure of similarity between any two query genes can be determined [5, 7]. Thus, individual genetic interactions can be aggregated into biological networks, which can provide valuable insights into the organization of the cell and subcellular processes [8]. Additionally, genes found to exhibit genetic interactions can be attractive drug targets. For example, an emerging therapeutic strategy for cancer is to induce selective lethality in tumors by exploiting interactions between driver mutations and specific drug targets [6].

This method described here differs from previous *S. cerevisiae* genetic interaction screening techniques in that the scale has been increased to include 6144 yeast colonies per plate. This number is important as it allows for the entire *S. cerevisiae* genome (~6000 genes) to be assayed against one query gene simultaneously, which in turns reduces the time and plate requirements for an interaction screen by up to fourfold. Here, we describe a method of generating double mutant knockouts, quantitatively assaying the resulting genetic interactions from fitness measures of colony size and an automated means of analyzing the data.

2 Materials

2.1 Strains and Plasmids for Generation of Query Strains

1. Haploid starter strain: MATα; his3Δ1; leu2Δ0; ura3Δ0; LYS2+; can1::STE2pr-HIS5; lyp1Δ::STE3pr-LEU2.
2. Diploid starter strain: MATa/MATα can1Δ::MFA1pr- HIS3/can1Δ::MFA1pr-HIS3-MFα1pr-LEU2 his3Δ1/his3Δ1 leu2Δ0/leu2Δ0 ura3Δ0/ura3Δ0 met15Δ0/met15Δ0 LYS2/lys2Δ0 LYP1/lyp1Δ0 [9] pFA6a-natMX6 (*see* **Note 1**).

2.2 Drug Concentrations

1. Geneticin (G418) 200 mg/mL.
2. Nourseothricin (NAT) 200 mg/mL.
3. L-Canavanine (CAN) 100 mg/mL.
4. *S*-(2-Aminoethyl)-L-cysteine hydrochloride (S-AEC) 100 mg/mL.

2.3 Transformation Media and Solutions

1. 40% glucose w/v.
2. 50% PEG 3350 w/v. Mix on stir plate overnight.
3. Zymolase 100T.
4. 1 M lithium acetate (LiAc).

5. YPAD media: (Per 1 L) 10 g yeast extract, 20 g peptone, 0.125 g adenine hemisulfate. Autoclave. Add 50 mL 40% glucose.
6. Competent cell freezing media: Add 5% v/v glycerol and 10% v/v DMSO to YPAD. Filter-sterilize.
7. YPAD + NAT plates: (Per 1 L) 10 g yeast extract, 20 g peptone, 20 g agar, 0.125 g adenine hemisulfate, 50 mL 40% glucose, 1 mL NAT.
8. Transformation mix: (Per one reaction) 260 µL 50% PEG 3350, 50 µL salmon sperm DNA 10 mg/mL, 36 µL 1 M LiAc. Filter sterilize.
9. Zymolase mix: 6 mg zymolase 100T and 10 mL double distilled water (DDW). Filter sterilize. Store at −20 °C.
10. Gene specific transformation primers (*see* **Note 2**).

2.4 Transformation Media for DAmP Essential Strains

1. Potassium acetate, 500 mg/mL filter sterilized.
2. CSM-Ura-Trp amino acid powder (Sunrise Science): adenine hemisulfate (10 mg/L), L-arginine (50 mg/L), L-aspartic acid (80 mg/L), L-histidine hydrochloride monohydrate (20 mg/L), L-isoleucine (50 mg/L), L-leucine (100 mg/L), L-lysine hydrochloride (50 mg/L), L-methionine (20 mg/L), L-phenylalanine (50 mg/L), L-threonine (100 mg/L), L-tyrosine (50 mg/L), L-valine (140 mg/L).
3. SPO media: (Per 1 L) Flask 1 = 820 mL DDW; Flask 2 = 0.5 g CSM-Ura-Trp amino acid powder, 2.5 mL 20 mM uracil stock, 2.5 mL 20 mM tryptophan stock, 163 mL DDW. Autoclave each flask separately. Mix flask. Cool media to approximately 60 °C then add 20 mL potassium acetate solution.
4. DAmP drop-out mix: 3 g adenine, 2 g alanine, 2 g asparagine, 2 g aspartic acid, 2 g cysteine, 2 g glutamine, 2 g glutamic acid, 2 g glycine, 2 g histidine, 2 g inositol, 2 g methionine, 0.2 g para-aminobenzoic acid, 2 g phenylalanine, 2 g proline, 2 g serine, 2 g threonine, 2 g tryptophan, 2 g tyrosine, 2 g uracil, 2 g valine.
5. DAmP selection plates (SD + MSG + NAT + S-AEC + CAN –LEU –ARG –LYS): (Per 1 L) Flask 1 = 20 g agar, 820 mL DDW; Flask 2 = 1.7 g yeast nitrogen base without amino acids and without ammonium sulfate, 2 g DaMP drop-out mix (–LEU –ARG –LYS), 1 g monosodium glutamic acid, 100 mL DDW. Autoclave Flask 1. Filter sterilize Flask 2. Mix Flask 1 and 2. Add 50 mL 40% glucose, 1 mL NAT, 0.5 mL CAN, 0.5 mL S-AEC.
6. 2 M sorbitol filter sterilized.
7. DAmP zymolase Mix: (Per 1 mL) 500 µL 2 M sorbitol, 10 µL 1 M Tris pH 7.5, 0.5 mg zymolase 100T (*see* **Note 3**).

2.5 Synthetic Genetic Array (SGA) Screen

2.5.1 Strains

1. Haploid query strains: MATα; his3Δ1; leu2Δ0; ura3Δ0; LYS2+; can1::STE2pr-HIS5; lyp1Δ::STE3pr-LEU2; XXX::NatMX).
2. Library strains: MATa; his3Δ1; leu2Δ0; ura3Δ0; met15Δ0; LYS2+; CAN1+; LYP1+ YYY::KanMx.
3. DaMP haploid strains: MATa; his3Δ1 leu2Δ0 ura3Δ0 met15Δ0 CYH2+ YYY::KanMx.
4. Wild-type HO.

2.5.2 Media

1. YPAD: (Per 1 L) 10 g yeast extract, 20 g peptone, 20 g agar, 0.125 g adenine hemisulfate. Autoclave. Add 50 mL 40% glucose w/v (*see* **Note 4**).
2. DS (Diploid Selection media): (Per 1 L) 10 g yeast extract, 20 g peptone, 20 g agar, 1 mL G418, 1 mL NAT (*see* **Note 5**).
3. SPO (sporulation media): (Per 1 L) Flask 1 = 820 mL DDW; Flask 2 = 0.5 g CSM-Ura-Trp amino acid powder, 2.5 mL 20 mM uracil stock, 2.5 mL 20 mM tryptophan stock, 163 mL DDW. Autoclave each flask separately. Mix both flask. Cool media to approximately 60 °C then add 20 mL potassium acetate solution (*see* **Note 6**).
4. HS (haploid selection media) (–HIS –LYS –ARG): Flask 1 = 20 g agar, 850 mL DDW; Flask 2 = 6.7 g yeast nitrogen base without amino acids, 2 g drop-out mix, 100 mL DDW. Autoclave both flasks. Mix both flask together and add 50 mL 40% glucose, 0.5 mL CAN, 0.5 mL 100 mg/mL S-AEC (*see* **Note** 7).
5. Drop-out mix: 3 g adenine, 2 g alanine, 2 g asparagine, 2 g aspartic acid, 2 g cysteine, 2 g glutamine, 2 g glutamic acid, 2 g glycine, 2 g inositol, 2 g isoleucine, 10 g leucine, 2 g methionine, 0.2 g para-aminobenzoic acid, 2 g phenylalanine, 2 g proline, 2 g serine, 2 g threonine, 2 g tryptophan, 2 g tyrosine, 2 g uracil, 2 g valine.
6. SM (single mutant selection media) (+MSG –HIS –LYS –ARG): Flask 1 = 20 g agar and 850 mL DDW. Flask 2 = 1.7 g yeast nitrogen base without amino acids and without ammonium sulfate, 2 g drop-out mix, 1 g monosodium glutamic acid, and 100 mL DDW. Autoclave Flask 1 and filter sterilize Flask 2. Mix both flasks together. Add 50 mL 40% glucose, 1 mL G418, 0.5 mL CAN, 0.5 mL S-AEC (*see* **Note 8**).
7. DMS (double mutant selection media) (+MSG –HIS –LYS –ARG): Flask 1 = 20 g agar and 850 mL DDW. Flask 2 = 1.7 g yeast nitrogen base without amino acids and without ammonium sulfate, 2 g drop-out mix, 1 g monosodium glutamic acid, and 100 mL DDW. Autoclave Flask 1 and filter

sterilize Flask 2. Mix both flasks together. Add 50 mL 40% glucose, 1 mL G418, 1 mL NAT, 0.5 mL CAN, 0.5 mL S-AEC.

3 Methods

3.1 Designing Transformation Products

1. Custom oligos used to build transformation product were constructed by Integrated DNA Technology (IDT) (*see* **Note 1**).
2. Combine 10 μL 2× iProof HF (Biorad), 0.25–0.5 μM each primer, 3% DMSO, 100 ng pFA6a-natMX6 DNA. Bring volume to 20 μL.
3. PCR cycle: 98 °C 3 min; 34 cycles of 98 °C 10 s, 60 °C 30 s, 72 °C 40 s; 72 °C 10 min; 4 °C hold.
4. Run 5 μL of PCR product on 1% TAE gel for 45 min at 80 V.

3.2 Making Yeast Query Competent Cells

1. Inoculate a single colony of haploid starter strain for nonessential genes and diploid starter strain for essential genes [10] into YPAD and grow overnight at 30 °C with shaking approximately 200–220 rpm [11].
2. The next day inoculate 5 mL of prewarmed YPAD with each overnight culture at a starting OD_{600} 0.2 and grow to 0.8–1.5 (approximately 5 h).
3. Harvest cells by centrifugation at 1430 × *g* for 5 min. Remove supernatant.
4. Resuspend cells in 0.5 volume of sterile DDW.
5. Centrifuge at 1430 × g for 5 min (repeat **steps 4** and **5**).
6. Resuspend cells in 50 μL (per 5 mL of yeast culture) in competent cell freezing media.
7. Cryopreserve cells in a styrofoam freezing or Mr. Frosty (Nalgene) container that allows the cells to freeze slowly in a −80 °C freezer (cells can be used for several months).

3.3 Cell Transformation for Essential and Nonessential Genes

1. Thaw competent cells quickly in a 37 °C water bath.
2. Centrifuge at 1430 × g for 5 min. Remove supernatant.
3. Add 346 μL of transformation mix. Mix well.
4. Add 15 μL of transformation product. Mix well.
5. Incubate in 42 °C water bath for 1 h.
6. Centrifuge at 1430 × g for 5 min. Remove supernatant.
7. Add 1 mL YPAD media to each transformant.
8. Incubate at 30 °C for 1–2 h 220 rpm.

9. Centrifuge at 1430 × g for 5 min. Remove 800 μL of supernatant and resuspend in residual 200 μL.
10. Plate all 200 μL of transformation onto YPAD +NAT plates.
11. Incubate at 30 °C for 2–3 days.

3.4 Validate Strains (Colony PCR)

1. Pick single colonies from cell transformation plates and place into 20 μL zymolase mix.
2. Lyse cells by incubating at 37 °C 30 min, 95 °C 10 min, 4 °C 2 min.
3. Spin briefly to pellet cell debris.
4. Combine 10 μL 2× iProof HF (Biorad), 0.25–0.5 μM each confirmation primer, 3% DMSO, 2 μL cell lysate. Bring volume to 20 μL (*see* **Note 9**).
5. PCR cycle: 98 °C 3 min; 34 cycles of 98 °C 10 s, 60 °C 30 s, 72 °C 10 s; 72 °C 10 min; 4 °C hold.
6. Run 20 μL of PCR product on 1% TAE gel for 45 min at 80 V.

3.5 Yeast DAmP Protocol for Making Essential Strains

1. Start 3 mL overnight culture in YPAD for each verified colony from Subheading 3.3 [12].
2. Transfer 1 mL of saturated overnight culture to 2 mL 96 deep-well plate.
3. Centrifuge cells at 1430 × g for 5 min. Discard supernatant.
4. Resuspend cells in 1 mL SPO media and cover plate with breathable membrane.
5. Incubate at 30 °C for 5 days at 225 rpm.
6. Transfer 100 μL of sporolating cells to 1.5 mL tube.
7. Centrifuge cells at 1430 × g for 5 min. Discard supernatant.
8. Resuspend cells in 100 μL DAmP zymolase mix.
9. Incubate at 30 °C for 45 min.
10. Add 1 mL 1 M sorbitol and place on ice.
11. Plate 150 μL of cells on DAmP selection plates.
12. Once single colonies grow, validate strains with colony PCR (Subheading 3.4).

3.6 Synthetic Genetic Array (SGA)

3.6.1 Preparing Query Lawn

1. Inoculate 5 mL YPAD with single colony of the query strain (*see* **Note 10**).
2. Grow overnight at 30 °C with 225 rpm shaking to saturation.
3. Vortex sample. Spread 5 mL saturated culture evenly over prewarmed YPAD +NAT plate.
4. Incubate plate for 2 days at 30 °C.

5. Pin lawn into 6144 format on YPAD +NAT plates. Default pinning settings unless specified (*see* **Note 11**).
6. Place plates into a resealable plastic bag (Ziplock) and incubate overnight at room temperature [13]

3.6.2 Preparing Library (See Note 12)

1. Pin frozen library stocks onto four YPAD +G418 plates in 1536 format.
2. Place plates into a resealable plastic bag and incubate at 30 °C overnight in incubator.

3.6.3 Mating

1. Pin query strains onto YPAD plates.
2. Pin library strains over query strains.
3. Place plates into a resealable plastic bag and incubate overnight at room temperature (*see* **Note 13**).

3.6.4 Diploid Selection

1. Pin mating plates onto DS plates (*see* **Note 14**).
2. Place plates into a resealable plastic bag and incubate 2 days at room temperature.

3.6.5 Sporulation

1. Pin DS plates onto SPO plates (*see* **Note 15**).
2. Place plates into a resealable plastic bag and place in dark area for 5 days at room temperature.

3.6.6 Haploid Selection 1

1. Pin SPO plates onto HS plates.
2. Place plates into a resealable plastic bag and incubate 2 days at room temperature.

3.6.7 Haploid Selection 2

1. Pin HS1 plates onto fresh HS plates.
2. Place plates into a resealable plastic bag and incubate overnight at room temperature.

3.6.8 Single Mutant Selection

1. Pin HS2 plates onto SM plates.
2. Place plates into a resealable plastic bag and incubate overnight at room temperature.

3.6.9 Double Mutant Selection

1. Pin SM plates onto DM plates.
2. Begin imaging plates (*see* **Note 16**).

3.7 Digital Imaging

A Canon E0S Rebel T3i camera (18 megapixels) with an 18–55 mm lens is used to take color digital photographs (raw) of every double mutant selection (DMS) plate. With the focal length set to 55 mm and the f-stop at 5.6, the images were taken at a distance of 44.45 cm (17.5 in.) by mounting the camera on a KAISER camera stand (Germany). The resulting images have a resolution of 240 dpi. The position of the plates were fixed into a

black, felt covered, notched platform attached to the camera stand. Illumination is provided by two fixed lamps (26 W, 120 vac, 60 Hz, 300 mA, 1635 lumens) spaced 8 in. from nylon white tent (20 × 20 × 20 in.) which serves as a light modifier around the camera station.

3.8 Data Analysis

Interaction scores are calculated as the deviation in observed fitness (colony size), of a double knockout strain from an expected fitness due two independent single gene knockouts. Colony sizes are detected and scored with a suite of custom algorithms written in MATLAB. The "Colony Analyzer Toolkit" (https://github.com/brazilbean/Matlab-Colony-Analyzer-Toolkit allows for systematic spot size detection of imaged EMAP screens in any array format (e.g., 6144) by automatically aligning a grid, subtracting the background intensity, and calculating the resulting colony area [14]. Due to systematic effects of nutrient availability, uneven agar surface, and subplate growth effects, spatial normalization of the colony sizes is important. The normalized spot sizes are then further processed in an additionally suite of MATLAB tools (http://sourceforge.net/projects/emap-toolbox/) which allow for filtering of noisy strains, linkage disequilibrium filtering, and ultimately calculation of interaction scores (S-scores) [10].

3.8.1 Image Processing and Spot Size Detection

1. Set image directory and find all plate images (*see* **Note 17**).

```
>> imagedir = 'images/';
>> files = dirfile(imagedir, '*.RAW');
```

2. Define parameters for image analysis.

```
>> params = { ...
>> 'parallel', true, ...
>> 'verbose', true, ...
>> 'grid', OffsetAutoGrid(), ... default
>> 'threshold', BackgroundOffset() };
```

3. Spot check the default parameters on an image.

```
>> analyze_image(files{1}, params{:});
```

4. Visualize the thresholded image to ensure it aligns to the grid.

```
>> view_plate_image(files{i}, 'applyThreshold',
true)
```

5. If satisfied with automatic results, perform image analysis on all the plate images.

```
>> analyze_directory_of_images(imagedir, params{:});
```

6. Manually, inspect all the grid alignments, noting potentially misaligned images.

```
>> out = manual_binary_inspection(imagedir );
```

7. For misaligned images, manually specify the corner colonies during analysis.

```
>> bad_images = find([out{:}] == 'n');
>> for i=1:size(bad_images,2)
>> analyze_image(files{bad_images(i)}, params{:},
   'grid', ManualGrid('dimensions', [64 96]) );
>> end
```

3.8.2 Compute S-Scores

1. Load in observed colony sizes from image analysis above.

```
>> cs = load_colony_sizes(imagedir );
```

2. Take mean across replicates and reshape matrix (*see* **Note 18**).

```
>> cs = squeeze(mean(reshape(cs, [5 5 4 1536]),1));
```

3. Apply border and within plate spatial correction.

```
>> cs_normalized = apply_correction(cs, 'dim', 3,
   SpatialBorderMedian(), PlateMode() );
```

4. Compute single mutant fitnesses.

```
>> cs_single_mutant_fitness =
   NaN(size(cs_normalized, 3), 1);
>> for i=1:size(cs_single_mutant_fitness,1)
>> array_gene = cs_normalized(:,:,i);
>> cs_single_mutant_fitness(i) =
   nanmedian(array_gene(:));
>> end
```

5. Calculate error estimates on normalized colony sizes.

```
>> cs_error =
   compute_error_estimates(cs_normalized, cs);
```

6. Calculate genetic interaction S-scores.

```
>> [cs_s_scores, cs_s_var] =
   compute_s_scores(cs_normalized, cs_error,
   cs_single_mutant_fitness);
```

4 Notes

1. Transform pFA6a-natMX6 into DH5α cells following standard protocol. Isolate plasmid DNA. Make glycerol stock of transformation and store at −80 °C.
2. All transformation products (NAT cassettes), confirmation primer sequences, and their respective product sizes are found on the Saccharomyces Genome Deletion Project website (www-sequence.stanford.edu/group/yeast_deletion_project/). We made slight changes to the original protocol. The NAT deletion cassette was constructed for each gene by PCR using custom gene specific primers and pFA6a-natMX6 DNA which provides a dominant selectable marker, nourseothricin-resistance (clonNat) [15]. The 60 bp UPTAG primers that we had built contained approximately 42 mers directly upstream of each gene specific open reading frame, including the ATG and a common tag-priming site (CGTACGCTGCAGGTCGAC) which has sequence homology to the 5′ region found in both the Kan gene in the *kanMX4* and the NAT gene in the *natMX6* modules. The 60 bp DNTAG primers contain approximately 42 mers directly downstream of each open reading frame, including the stop codon and a common tag priming site (ATCGATGAATTCGAGCTCG) which has sequence homology to the 3′ downstream from the Kan gene in the *kanMX4* and the NAT gene in the *natMX6* modules [6, 16].
3. Zymolase mix can be aliquoted and stored at −20 °C for several months.
4. Aliquot 50 mL of media to each plate. Plates must be extremely level to pin 6144 format. Although there are 6144 pin pads, we have found them to be extremely inflexible to any slight disparity in level. Also, plates must be dry and free of any water drops.
5. G418 and NAT are used to enrich for diploid strains carrying both markers. Cool media to approximately 60 °C before drugs are added.
6. Meiosis and sporolation is initiated by nitrogen starvation in the media.
7. In order to select for the proper ploidy and mating type, haploid selection was completed in two steps. The *CAN1* gene encodes for sensitivity to toxic arginine analog canavanine and *LYP1* gene is sensitive to S-AEC. The addition of canavanine and S-AEC to the media selects against the parental diploid cells, which are heterozygous for these genes. To facilitate proper mating type, the *S. pombe his5* (the complement of the *S. cerevisiae his3* mutation) mating type-specific promoter (STE2pr), drives the transcription of the essential metabolic

gene *HIS3* only in the haploid mating type Mat a and allows the cells the ability to grow in media deficient of histidine and constitutively expressing resistance markers.

8. Monosodium glutamic acid (MSG) is added to the medium as the nitrogen source because ammonium sulfate can impair the function of G418 and NAT [17].
9. Cell lysates can be stored in −20 °C and reused for at least a month.
10. For each query strain, three biological replicates were prepared along with Matα wild-type HO strain as a control. The site-specific endonuclease known as, homothallic switching (HO), allows the yeast to switch between mating types by cleaving the mat locus on chromosome III. By knocking this gene out, the Matα wild-type HO strain will not be able to initiate interconversion of mating type [18].
11. All pinning steps were completed using a Singer Rotor HDA. All rotor plates were purchased through VWR (75780–348). All 6144 plates were pinned using four 1536 short pin pads (Singer). Singer rotor HDA default settings: 1536-to-6144 for query mating (Offset: Random; Pin Pressure: 58%; Speed: 19 mm/s; Overshoot: 2 mm; Radius: 0.3 mm; Repeat pin: 1; Recycle: ON); 1536-to-6144 library mating and 6144-to-6144 replicating (Offset: Random; Pin Pressure: 58%; Speed: 19 mm/s; Overshoot: 2 mm; Radius: 0.3 mm; Repeat pin: 1; Recycle: OFF).
12. SGA library plates were reconfigured to combine the entire MatA knockout collection (4944 strains) (Dharmacon) with the DaMP haploid essential collection (842 genes) (Dharmacon) into one 6144 library plate.
13. One set of four 1536 library plates can mate with four 6144 query plates. Make sure there are enough library plates to cover all matings. Strains grown in 6144 format overgrow at 30 °C overnight. Therefore, all pinning steps in 6144 format were incubated overnight at room temperature in resealable plastic bags. G418 and NAT were used to enrich for the diploid strains.
14. G418 and NAT were used to enrich for diploid strains.
15. Cells do not divide on SPO media. You can increase pressure to maximize cell transfer.
16. We begin imaging plates at 3 h. We notice the colony sizes are negligible before that. We take our plate images with the lids off and upside down. Yeast colonies grow like a small mound. This can reflect light differently and affect the image intensity of the spot.

17. If the raw images are compressed prior to analysis, for example into jpeg format, the resulting regular expression string must be modified to reflect the file type used. For example, if jpeg files are used change the code to ">> files = dirfile(imagedir, '*.jpeg');".
18. The reshape function must reflect the experimental design and plate nomenclature. For example, the call here corresponds to data from five image replicates across five array sets across four conditions. Thus, reshaping 100 images into these categories and taking the mean results in a data set with 5 array sets × 4 conditions × 1536 spots.

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References

1. Beyer A, Bandyopadhyay S, Ideker T (2007) Integrating physical and genetic maps: from genomes to interaction networks. Nat Rev Genet 8:699–710
2. Collins SR, Miller KM, Maas NL, Roguev A, Fillingham J, Chu CS et al (2007) Functional dissection of protein complexes involved in yeast chromosome biology using a genetic interaction map. Nature 446:806–810
3. Pan X, Ye P, Yuan DS, Wang X, Bader JS, Boeke JD (2006) A DNA integrity network in the yeast Saccharomyces cerevisiae. Cell 124:1069–1081
4. Tong AHY, Lesage G, Bader GD, Ding H, Xu H, Xin X et al (2004) Global mapping of the yeast genetic interaction network. Science 303:808–813
5. Costanzo M, VanderSluis B, Koch EN, Baryshnikova A, Pons C, Tan G et al (2016) A global genetic interaction network maps a wiring diagram of cellular function. Science 353. https://doi.org/10.1126/science.aaf1420
6. Srivas R, Shen JP, Yang CC, Sun SM, Li J, Gross AM et al (2016) A network of conserved synthetic lethal interactions for exploration of precision Cancer therapy. Mol Cell 63:514–525
7. Myers CL, Barrett DR, Hibbs MA, Huttenhower C, Troyanskaya OG (2006) Finding function: evaluation methods for functional genomic data. BMC Genomics 7:187
8. Shannon P, Markiel A, Ozier O, Baliga NS, Wang JT, Ramage D et al (2003) Cytoscape: a software environment for integrated models of biomolecular interaction networks. Genome Res 13:2498–2504
9. Schuldiner M, Collins SR, Thompson NJ, Denic V, Bhamidipati A, Punna T et al (2005) Exploration of the function and organization of the yeast early secretory pathway through an epistatic miniarray profile. Cell 123:507–519
10. Collins SR, Roguev A, Krogan NJ (2010) Quantitative genetic interaction mapping using the E-MAP approach. Methods Enzymol 470:205–231
11. Gietz RD, Schiestl RH (2007) High-efficiency yeast transformation using the LiAc/SS carrier DNA/PEG method. Nat Protoc 2:31–34
12. Schuldiner M, Collins SR, Weissman JS, Krogan NJ (2006) Quantitative genetic analysis in Saccharomyces cerevisiae using epistatic miniarray profiles (E-MAPs) and its application to chromatin functions. Methods 40:344–352
13. Bean GJ, Jaeger PA, Bahr S, Ideker T (2014) Development of ultra-high-density screening tools for microbial "omics". PLoS One e85177:9
14. Collins SR, Schuldiner M, Krogan NJ, Weissman JS (2006) A strategy for extracting and analyzing large-scale quantitative epistatic interaction data. Genome Biol 7:R63
15. Tong AHY, Boone C (2006) Synthetic genetic array analysis in *Saccharomyces cerevisiae*. Methods Mol Biol 313:171–192

16. Bandyopadhyay S, Mehta M, Kuo D, Sung M-K, Chuang R, Jaehnig EJ et al (2010) Rewiring of genetic networks in response to DNA damage. Science 330:1385–1389
17. Tong AH, Evangelista M, Parsons AB, Xu H, Bader GD, Pagé N et al (2001) Systematic genetic analysis with ordered arrays of yeast deletion mutants. Science 294:2364–2368
18. Haber JE (2012) Mating-type genes and MAT switching in *Saccharomyces cerevisiae*. Genetics 191:33–64