I) Materials

Yeast media, stock solutions and drugs

1) YEPD media (for 1L, 25 plates): 120mg adenine, 10g yeast extract, 20g peptone, 20g bacto agar, 950ml distilled water
*Autoclave for 45 minutes and cooled to 65°C
*Add 50ml of 50% sugar stock
*Pour media into square OmniTrays (Nunc, 242811)

2) Synthetic media (for 1L, 25 plates): 1.7 g yeast nitrogen base without amino acids and ammonium sulfate (BD Difco), 1.0 g MSG (L-glutamic acid sodium salt hydrate, Sigma), 20 g bacto agar (for solid media only), 850 ml distilled water
*Autoclave for 45 minutes and cooled to 65°C
*Add 50ml of 50% sugar stock, 100ml of 10X amino acid drop out and 1ml of appropriate drug stock solution if required (See steps 3 and 4).
*Pour media into square OmniTrays (Nunc, 242811)

3) Amino acid supplement powder stock: 3g adenine, 2g uracil, 2g inositol, 0.2g para-aminobenozoic acid, 2g alanine, 2g arginine, 2g asparagine, 2g aspartic acid, 2g cysteine, 2g glutamic acid, 2g glutamine, 2g glycine, 2g histidine, 2g isoleucine, 2g leucine, 2g lysine, 2g methionine, 2g phenylalanine, 2g proline, 2g serine, 2g threonine, 2g tryptophan, 2g tyrosine, 2g valine.
*Drop out mix is created as a combination of all the above amino acids minus the appropriate selectable supplement. Mix 2g of dry stock is used per litre of medium.

4) Sugar stocks (Autoclaved): i) 40% Dextrose (Glucose, Fisher)
ii) 40% Galactose (Fisher)
iii) 40% Raffinose (Fisher)

5) Sporulation amino acid mix*: 2g histadine, 1g leucine, 2g lysine, 2g uracil,
*0.1g of amino-acid supplement powder mix is used per litre of sporulation medium

6) Drug stocks used for selection (filter sterilized and stored at 4°C):

i) Canavinine (L-canavanine sulfate salt, Sigma C-9758)	(100mg/ml)
ii) cloNAT (Nourseothricin, Werner BioAgents, Jena, Germany)	(100mg/ml)
iii) Thialysine (S-(2-aminoethyl)-L-cysteine hydrochloride, Sigma A-2636)	(100mg/ml)

* 1ml of each drug is used for 1L of media

Robotics and Equipment:

1) Array Pinning: i) VirTek array pinning robot ii)384 floating pinhead iii)1536 floating pinhead

2) High Resolution Camera: Cannon Powershot G2 (>4X megapixels) mounted on a stand

3) Serial Spot Dilution: i) Percision XS (BioTek Instruments Inc.) ii) Robotic pipette tips [1-50µl] (Fisher scientific, 02-707-344)

Strains:

1) Wild type query genotype (Y8835):
S288C, *MATα ura3*Δ::*NAT*^{R} *can1*Δ::*STE2pr-Sp_HIS5 lyp1*Δ *his3*Δ1 *leu2*Δ0 *ura3*Δ0 *met15*Δ0 *LYS2+*

2) SDL query genotype:
S288C, *MATα yfg*Δ::*NAT*^{R} *can1*Δ::*STE2pr-Sp_HIS5 lyp1*Δ *his3*Δ1 *leu2*Δ0 *ura3*Δ0 *met15*Δ0 *LYS2+*
*yfg is any gene of interest

3) GST overexpression array genotype (BY4741):

S288C, *MAT*a *his3*Δ1, *leu2*Δ0, *met15*Δ0, *ura3*Δ0 + pEGH plasmid (*pGAL1/10-6XHIS-GST-ORF-URA3*)
*ORF is the open reading frame for each gene in the yeast genome. 5380 ORFs are present on the array ordered on 19 plates. The GST overexpression yeast collection can be purchased from Open Biosystems

Confirmations

1) Plates:

i) 96-well sterile corner notch microplates (Costar, 3791) and lids (Costar, 3930)

ii) LB+Amp plates (for 1L, 40 plates): 10g Bactotryptone (BD Biosciences, 211699), 5g Yeast Extract (BD Biosciences, 212720), 5g Sodium Chloride (Bioshop, SOD001.5), 500µl Sodium Hydroxide (VWR, VW3247-1), 15g bactoagar (BD Biosciences, 214030, For plates only) and 1L distilled water

*Autoclave for 45 minutes and cool to 65°C. Add 1ml of Ampicillin (100 mg/ml stock) and pour media into sterile round plates

2) HTP plasmid DNA extraction: Nucleospin 96-well mini-prep kit (Clonetech)

3) Stock solutions for HTP transformations:

i) Lithium Acetate (LiOAc, 1M, filter sterilized)

ii) Polyethyleneglycol (PEG, 50% solution, filter sterilized)

iii) Carrier DNA (salmon sperm DNA, 2mg/ml)

4) HTP spot dilution and growth curve liquid media

1) SD media: Prepared as described in 2.1, using glucose as the sugar source, without agar

2) S media (for 1L): 1.7 g Yeast Nitrogen Base (without amino acids and ammonium sulfate) is dissolved in 1L of distilled water and autoclaved for 45min.

II) Methods

Synthetic Genetic Array (SGA) methodology for HTP Synthetic Dosage Lethality (SDL) screening (Plate-based assay, scoring colony fitness)

1) Lawn preparation: Grow the query strain in 10ml overnight culture in YEPD liquid media. Centrifuge overnight culture and resuspend in 2ml YEPD liquid media. Pour the query strain culture in YEPD plates and grow overnight at 30°C.

2) Array preparation: The array is maintained in 384 format and is converted to 1536 format using the Virtek Robot. Replicate the overexpression array in 1536 format onto fresh SD-U plates and grow cells for two days at 30°C.

3) Mating: Pin the query strain lawn onto fresh YEPD plates and pin the freshly grown 1536 overexpression array over top of the newly pinned YEPD plates. Incubate mating plates overnight at room temperature.

4) *MAT*a/α diploid selection: Replicate the resulting *MAT*a/α zygotes in 1536 format onto SD-Uracil+cloNAT (SD-U+N) to select for diploids that contain markers from both the query deletion strain (NAT^R) and overexpression strains on the array (*URA3*). Incubate diploid selection plates at 30°C for 2 days. Repeat the diploid selection step, this time growing diploid selection plates overnight at 30°C.

5) Sporulation: Pin diploid cells onto sporulation medium (See section 2.1, step 5) containing 25µg/ml cloNAT to avoid contamination and incubate plates 5-10 days. Sporulation must be performed at 22°C - 24°C for maxium efficiency and plates can be stored for up to 4 months at 4°C.

6) Haploid Selection:

Selecting *MAT*a meiotic progeny: Pin spores onto haploid selection plates (SD-Ura/Arg/His/Lys+ canavanine + thialysine +cloNAT) and incubate cells for 2-3 days at 30C. Repeat the haploid selection step for a second round of selection and incubate plates for 1 day at 30°C.

7) Induction of gene overexpression: Pin *MAT*a haploids onto both glucose (SD-Ura/Arg/His/Lys+ canavanine + thialysine +cloNAT) and galactose (inducing overexpression) plates (SG-Ura/Arg/His/Lys+ canavanine + thialysine +cloNAT) and incubate cells at 30C for 2 days (glucose) and 3 days (galactose) respectively.

8) Image capture: Take high resolution pictures of induced and uninduced final plates for image analysis.

Confirmations using serial spot dilutions

Secondary growth assay are required for confirming putative genetic interactions identified from SDL screens using SGA. Here we use serial spot assays for secondary growth assessment.

HTP miniprep of plasmids from bacterial glycerol stocks

1) Streak out bacterial glycerol stocks of plasmids expressing each ORF identified in the genome-wide screens using LB+Amp plates.
2) Inoculate single bacterial colonies in 1ml liquid LB+Amp medium and grow at 37C in deep well 96-well sterile dishes for 2 days.
3) Centrifuge bacterial cultures at 6000rpm for 10 min and discard supernatant.
4) Extract the plasmids expressing each ORF using the Nucleospin high-throughput mini-prep kit (Clontech). Elute DNA in 100µl Elution Buffer supplied by the manufacturer.

HTP transformation of plasmids into control and query yeast strains

1) Set up 10ml overnight culture in YEPD liquid media for both wild type and mutant query strains
2) Make 300ml of diluted overnight culture at in YEPD liquid media at $O.D._{600}$~0.1 and grow on shaker at 30°C for 4-5 hrs (until culture reaches $O.D._{600}$~0.8).
3) Centrifuge log-phase cultures at 3000rpm for 3min and discard supernatant.
4) Wash pellet with 10ml of sterile distilled water.
5) Re-suspended washed pellets in 6ml of filter sterilized 0.1M LiOAc on ice and transfer to 50ml sterile falcon tubes
6)Spin down cells at 3000rpm for 3 min and discard supernatant.
7) Resuspend cells in 2ml sterile distilled water.
8) Add 14.4ml of 50% PEG, 3ml of 2mg/ml ssDNA and 2.2ml of filter sterilized LiOAc (1M)
9) Vortex briefly and use the multi-channel pipettor to dispense 200ul of the mixture into each well of a micto titre plate, already containing 2µl of minipreped plasmid DNA. Include a negative control (no DNA).
10) Mix competent cells and DNA thoroughly using the muti-channel pipettor and incubate plate at 30°C for 30min for recovery phase.
11) Heat shock cells by incubating the plate at 42°C for 30min
12) Centrifuge plate at 3000rpm for 2min and remove PEG mixture from the cells (pellet)
13) Dispense 200µl if sterile distilled water per well using the multi-channel pipettor and incubate on bench for 5min at 25°C to allow the cell pellet to go loose.
14) Resuspend cells well and dispense 20µl of the cultures from each column onto square plates containing SD-Ura+cloNAT and drag the solution (liquid/cells) in a straight line, taking up half the OmniTray squareplate.

15) Incubate plates at 30°C for 3-4 days for single colonies to appear on each streak

Automated spot dilutions to assess defects due to gene overexpression in control versus query yeast strains

1) Grow 200µl of overnight cultures in the appropriate liquid synthetic media (SD-Ura) for 2 days at 30°C in 96 well sterile plates.
2) Make 15-fold dilutions of saturated overnight cultures in liquid "S" media (See materials) using the Precision XS serial diluting robot or by hand.
3) Spot 5µl of serial dilutions onto non-inducing (SD-Ura+cloNat) and inducing (SG-U+cloNat) synthetic solid media.
4) Grow for 2 days (glucose plates) or 3 days (galactose plates) respectively
5) Capture digital images of final plates for image analysis.
6) Using ImageJ or any imaging software to calculate the average for the pixel intensity in a given area for all five dilutions and normalize it to the fitness defect scores of the mutant strains to calculate an expected toxicity in the mutant query. Calculate the difference between the observed and expected toxicity due to ORF overexpression (ε=Expected toxicity in kinase delete-observed toxicity in kinase delete). ε≥0 indicates no interaction, while ε<0 represents a higher observed toxicity in the kinase deletion strain. Assign confidence scores based on the standard deviation (SD). For example: 3X SD-high confidence, 2X SD-medium confidence and 1.5X SD-low confidence. Cross-confirm all spot assay data by eye by comparing overexpression outcomes in wild type and mutant queries. This generates a list of confirmed SDL interactions for the mutant query. As an alternative to using Image J, unbiased visual toxicity scores (between 1 to 5)can be assigned where 1 represents the strongest interaction (1spot grows) and 5 represents no interaction (all 5 spots grows).