Supplementary Information

For

A functional variomics tool for discovering drug resistance genes and drug targets

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Inventory of Supplemental Information

Supplementary Data

Figure S1. Building a variomic library. A. Making the promoter/terminator (or P/T) clone of *YFG* (Your Favorite Gene) using homology-mediated cloning. The vector plasmid pXP597 is a low-copy centromeric (CEN) plasmid derived from pRS416. It contains gateway recombination cloning sites attB1 and attB2, allowing convenient transfer of YFG to other vectors. **B.** Making the variomic library. Error-prone PCR products of *YFG* (*YFG**) and NotI-digested plasmid DNA of the P/T clone of the same gene are co-transformed into yeast cells of a corresponding haploid-convertible heterozygous diploid deletion mutant. The presumably mutagenized PCR products are cloned onto the plasmid via homologous recombination in vivo. **(Related to Figure 1)**

Figure S2. A general strategy for identifying drug resistance gene and alleles using the functional variomics technology. The whole process can be divided into three seperate stages as indicated. The process seems to be complicated with many steps. However, the first stage is not required for all screens. Once a pool of haploid cells is made, it can be used for screening many different drugs starting directly from the second stage. Many drugs can also be screened in parallel to increase the throughput. **(Related to Figure 1)**

Figure S3. Dominant, semi-dominant, or recessive phenotypes of rapamycin resistance alleles of *TOR1* and *TOR2*. BY4743**a**/α cells carrying centromeric plasmids of indicated genotypes grown in the presence or absence of rapamycin (50 ng/ml) and incubated at 30°C for 2 days. **(Related to Figure 2)**

Figure S4. AmB-resistance conferred by expressing wild-type *PMP3* from a centromeric plasmid. BY4743**a**/α cells carrying centromeric plasmids of indicated genotypes were grown in the presence or absence of AmB (4.0 μg/ml) and incubated at 30°C for 2 days. **(Related to Figure 3)**

Table S1. A summary of the yeast genome-wide variomic libraries. (Related to Figure 1)

Table S2. Barcode sequencing results of screening the variomic libraries for resistance genes. (Related to Figures 2 & 3)

Table S3. Rapamycin resistance mutations in *TOR1* and *TOR2.* (Related to Figure 2)

TOR1			***TOR2***		
Allele No.	Nucleotide substitution	Amino acid substitution	Allele No.	Nucleotide substitution	Amino acid substitution
1	C5663A C5916A	P1886H S1972R	1	A5459G T5847C G5924A	H1820R S1975N
2	C5663A T6133C	P1886H F2044L	2	A5800G T5936C	I1934V F1979S
3	C5916G	S1972R	3	A5923C	S1975R
4	G5915A	S1972N	4	T5925A A6152G	S1975R I2051S
5	G5915A A6006G	S1972N	5	T5935G	F1979V
6	G5915A A6006G	S1972N	6	A6112G	N2036D
7	T5900C T5927C	W1964R F1976S	7	T6121C	W2041R
8	T6133C	F2044L	8	T6121C	W2041R
9	T6133C	F2044L	9	T6121C	W2041R
10	T6133C T6234C	F2044L	10	A6137G	N2046S

Note: Mutations responsible for rapamycin-resistance are colored in blue (known) and red (Novel). These alleles were ordered according to the position of the first mutations they harbor.

Supplementary Procedure

Designing primers for constructing the variomic libraries. Four primers were designed for each gene using a customized algorithm derived from *Primer 3* (Rozen and Skaletsky, 2000). These were promoter forward (PF), promoter reverse (PR), terminator forward (TF), and terminator reverse (TR) primers (Fig. S1a). The PF/PR and TF/TR primer pairs were used to PCR-amplify a gene's promoter (~500 bp of 5'-UTR) and terminator (~500bp or 3'-UTR), respectively. A PF/TR pair was used in error-prone PCR for random mutagenesis.

For cloning the promoter and terminator PCR products into pXP597 and pXP688 using homology-mediated methods (Gibson et al., 2009; Li and Elledge, 2007), the PF and TR primers was each designed to contain an adaptor sequence at the 5' end that was either identical or complimentary to the ends of SmaI-digested vectors. They also each contained gene-specific sequence at the 3' end for PCR-amplification of target sequences. For each gene, the PR and TF primers were completely complimentary and both contained three parts: two ~20 bp promoter- or terminator-specific sequences flanking a NotI recognition site. Such a configuration permits simultaneous assembly of both the promoter and terminator fragments of a gene in tandem, with a NotI site between them, onto an SmaI-digested vector. This NotI site would later be used to linearize the resultant clone for constructing the variomic libraries. The sequences of all primers are available upon request.

All primers were synthesized in 96-well plates, with all 4 primers of the same gene in concordant wells in 4 different plates to facilitate 96-well PCR. Importantly, they were arranged largely according to gene sizes, with genes of similar sizes on the same 96-well plates to permit unification of error-prone PCR conditions across each plate. For the essential gene set, the 2nd well in row G was kept empty for all plates both as an identifier of plate orientation and as a negative control during experiments. For the non-essential gene set, the 2nd well in row H was kept empty for the same purposes. Each plate also had a unique empty well as plate identifier.

Constructing the promoter/terminator clones Each promoter or terminator fragment was first separately PCR-amplified in a 25 μl reaction in 96-well plates using Ex Taq (Takara) under high fidelity conditions. Each PCR reaction included: DNA polymerase (0.6 units), 1x DNA polymerase buffer, dNTP (0.2 mM each), primers (0.5 μM each), and yeast genomic DNA (4 ng/μl) isolated from BY4743**a/α**. The cycling conditions were: 94ºC for 4 min, 30 cycles of 94ºC for 30 sec, 55ºC or 58ºC for 30 sec, and 72ºC for 45 sec, followed by 72ºC for 7 min. 2 μl of each PCR product was examined with agarose gel electrophoresis to verify successful PCR. Equal volumes of promoter and terminator PCR products of each gene were combined. The PCR products were cloned onto SmaI-digested pXP597 or pXp688 using SLIC (Li and Elledge, 2007) or a T5 exonuclease/DNA polymerase-dependent method (Gibson et al., 2009) as discussed below.

With SLIC (Li and Elledge, 2007), combined PCR products were purified with a 96-well PCR purification kit (Promega) according to the manufacturer's instructions and eluted in 25 μl of elution buffer. In the meanwhile, the plasmid pXP597 was digested with SmaI at 25ºC for overnight and purified using a gel extraction kit (Qiagen). The PCR products were cloned onto SmaI-digested vector in a 96-well format using a modified SLIC protocol. First, a master reaction mixture was prepared and kept on ice. For each reaction, this contained 2 μl of 10 x BamHI buffer (NEB), 2 μl of 10 x BSA, 0.3 μl of T4 DNA polymerase (3 units/μl, NEB), and 3.7 μl of ddH_2O in a total volume of 8 μl. 2 μl of purified vector DNA (~50 ng/μl) and 10 μl of purified PCR products (~50 ng/μl each) were added into each well of a regular 96-well plate. 8 μl of the resection mixture was subsequently added to each sample, mixed by briefly shaking on a microplate orbital shaker, and incubated at 25ºC for 30 min. The reactions were stopped with dCTP (1 mM). Here generation of 5' single-stranded DNA overhangs by T4 DNA polymerase and annealing of the complementary single-stranded DNA overhangs were carried out simultaneously in the same reaction.

With the T5 exonuclease/DNA polymerase-based method (Gibson et al., 2009), combined promoter and terminator PCR products were directly used without purification. The plasmid pXP688 was digested with SmaI and gel purified. Each 20 μl DNA assembly cloning reaction was set up essentially as described (Gibson et al., 2009) with minor modifications. This included 4 μl of digested vector (10 ng/μl), 1 μl of combined PCR products (~50ng/μl each), and 15 μl of 1.33x reaction buffer that contained T5 exonuclease (Epicentre; 0.08 units/reaction) and Phusion DNA polymerase (NEB; 0.06 units/reaction) (Gibson et al., 2009). The reactions were carried out in 96-well plates at 50ºC for 20 min in a thermal cycler and subsequently sat on ice.

With both methods, 2 μl of each reaction was transformed into 20 μl of chemically competent cells of DH5a prepared according to the Inoue method (Inoue et al., 1990) in a 96-well format as described previously (Huang et al., 2008). Representative single colony transformants were picked and arranged back to their original 96-well plate positions in liquid LB plus carbenicillin (50 μg/ml). In our hands, the T5 exonuclease/DNA polymerase-based method was more efficient than SLIC and this had nothing to do with the different vectors used. Its experimental procedure was also simpler than that of SLIC because there was no need to purify the promoter and terminator PCR products and to stop the assembly reactions by adding dCTP.

Each promoter/terminator clone was first validated with colony-PCR using Ex Taq (Takara) and a pair of primers 5'CCAGGCTTTACACTTTATGCT3' and 5'CTGTTGGGAAGGGCGATC3' that anneal to the vector backbones. Each colony-PCR reaction of 15 μl was set up similarly to that described above but with minor modifications. 0.5 μl of each low-density bacterial culture (0.1-0.5 $OD600_{nm}$/ml in LB plus carbenicillin) of picked transformant single colonies instead of yeast genomic DNA was used as PCR templates. The cycling conditions were: 94ºC for 4 min, 30 cycles of 94ºC for 30 sec, 55ºC or 58ºC for 30 sec, and 72ºC for 1 min, followed by 72ºC for 7 min. 5 μl of each PCR reaction was examined with

agarose gel electrophoresis to verify the presence of inserts in each clone. For clones with and without inserts, the expected sizes of PCR products were ~1.4 kb and ~0.4 kb, respectively.

DNA Sequencing of promoter/terminator clones. Sequencing reactions were carried out at Cogenics Lark Inc., Lone Star Labs, and Beckman Coulter Genomics Inc. using the primer 5'CCAGGCTTTACACTTTATGCT3' that anneal to all vectors. The sequencing data were retrieved and analyzed using a customized analysis pipeline and compared to the sequenced *Saccharomyces* genome database (http://www.yeastgenome.org/). When necessary, a second or third clone was sequenced for a gene. Correct clones were consolidated into one set of promoter/terminator clones.

Constructing the variomic libraries. Error-prone PCR was carried out in 96-well plates using the PF/TR primer pairs, Ex Taq DNA polymerase (Takara), and genomic DNA templates isolated from the wild-type yeast BY4743**a**/α under mutagenic conditions. A 100 μl PCR reaction was set up for each gene with 10 μl of 10 x Ex Taq buffer, 8 μl of dNTP (2.5 mM each), 8 μl of primer mix (5 μM each), 4 μl of yeast genomic DNA (100 ng/μl), 1 μl of $MnCl_2$ (100-500 μM), 0.5 μl of Ex Taq DNA polymerase (5 units/μl), and 68.5 μl of ddH_2O. To reduce potential founder effects, this was split into four 25 μl PCR reactions. The PCR conditions were: 94ºC for 4 min, 35 cycles of (94ºC for 30 sec, 55ºC for 30 sec, 72ºC for ~1 min/kb), and 72ºC for 7 min. After PCR, all four reactions of the same gene were combined. 2 μl of each PCR product was examined with agarose gel electrophoresis.

In the meanwhile, about 1-2 μg of each promoter/terminator clone on the centromeric plasmid was digested with NotI in a 30 μl reaction at 37ºC for overnight. 1 μl of each digestion reaction was examined with agarose gel electrophoresis to verify completion of digestion. The rest of each reaction was incubated at 65ºC for 20 min to inactivate NotI, combined with error-

prone PCR products of the same gene, cleaned up with a 96-well PCR purification kit (Qiagen) according to the manufacturer's suggestions, and resuspended in 50 μl of ddH_2O.

For yeast transformation, the haploid-convertible heterozygous diploid deletion mutants(Pan et al., 2006) were first arranged in 96-well plates according to the positions of DNA samples to be transformed. These strains were grown in regular 96-well plates containing liquid YPD (100 μl/well) at 30ºC for overnight without shaking. Subsequently each culture was used to seed two 1.5 ml YPD cultures in 96-well deep well plates at a cell density of ~0.1 OD_{600nm}/ml. A single sterile glass bead (~5 mm in diameter) had already been placed inside each well to help agitation and to prevent cell precipitation during incubation. Cells were incubated at 30ºC with shaking at a speed of 180 rpm until reaching a density of ~0.5 OD_{600nm}/ml. Cells from both plates of the same strains were harvested, washed once with 100 μl of 0.1 M lithium acetate, combined, and transferred into a regular 96-well plate. Cells were once again spun down. Supernatants were carefully removed with a 12-channel pipette, leaving behind the competent cells in a pact volume of ~5 μl. In the meanwhile, a master yeast transformation mixture of PEG-3350 (66.7%, 75 μl/reaction), lithium acetate (1M, 15 μl/reaction), and herring sperm DNA (10 μg/μl, 10 μl/reaction) was prepared and put aside. 45 μl of each purified DNA samples (of NotI-digested promoter/ terminator clones and error-prone PCR products) was transferred to the 96-well plate containing competent yeast cells of the corresponding heterozygous diploid deletion mutants. Yeast cells were resuspended in the DNA solutions by shaking on an orbital microplate shaker. The transformation mixture was subsequently added (at 100 μl/reaction) and mixed with yeast cells and DNA by pipetting for 5-7 times. After incubation at 30°C for 30 min, DMSO was added (15 μl/well) into the transformation reactions. This was followed by incubation at 42°C for 15 min. After that, cells were spun down, resuspended in 5 mM $CaCl_2$ (50 μl/well), and incubated at room temperature for 5-10 min. Cells were once again spun down, resuspended in sterile ddH_2O (50 μl/well), and transferred to a 96 deep well plate containing

liquid SC-Ura (1 ml/well). 0.2 or 1 μl of each culture was immediately plated on solid SC-Ura to determine the number of primary alleles of each variomic library. The rest of each culture was incubated at 30°C for an overnight with shaking to allow for an initial round of amplification by >20- to 50-fold. Cells of the variomimc libraries were then spun down, resuspended in 15% glycerol (100 μl/well), and stored at -80°C as permanent stocks.

Pooling, amplifying, and converting the variomic libraries into haploid cells. Frozen stocks of all variomic libraries were thawed. An average of ~1 x 10^5 Ura^+ cells were taken from each library and mixed together. The resultant pool was grown in 1 liters of liquid SC-Ura at 30°C with shaking (180 rpm) until reaching a cell density of ~1.0 OD_{600nm} /ml. This resulted in the amplification of Ura^+ cells by ~30-fold. The resultant pool was aliquoted and stored at -80°C in 15% glycerol. An aliquot of 100 OD_{600nm} cells from the amplified pool was grown in another 1-liter liquid SC-Ura culture at 30°C until reaching a cell density of ~1.0 OD_{600nm}/ml. This resulted in further amplification of Ura^+ cells by about 10-fold. Cells were spun down, washed once in sterile water, resupended in 15% glycerol, aliquoted, and stored at -80ºC or sporulated. ~6 x 10^9 Ura^+ cells from the amplified pool were sporulated in 1 liter of liquid sporulation medium, subsequently inoculated into 1 liter of a liquid haploid selection medium (SC-Arg-His-Leu-Ura+G418+canavanine) (Pan et al., 2004) to select for haploid *MAT***a** $G418^R$ Ura^+ cells, and incubated with shaking at 30ºC until reaching a cell density of ~1.0 OD_{600nm}/ml. These haploid cells were either directly screened for drug resistance genes or stored as glycerol stocks at -80ºC.

Supplementary Reference

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