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Functional Profiling of the *Saccharomyces cerevisiae* Genome

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Functional profiling of the *Saccharomyces cerevisiae* genome

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Determining the effect of gene deletion is a fundamental approach to understanding gene function. Conventional genetic screens exhibit biases, and genes contributing to a phenotype are often missed. We systematically constructed a nearly complete collection of gene-deletion mutants (96% of annotated open reading frames, or ORFs) of the yeast *Saccharomyces cerevisiae*. DNA sequences dubbed ‘molecular bar codes’ uniquely identify each strain, enabling their growth to be analysed in parallel and the fitness contribution of each gene to be quantitatively assessed by hybridization to high-density oligonucleotide arrays. We show that previously known and new genes are necessary for optimal growth under six well-studied conditions: high salt, sorbitol, galactose, pH 8, minimal medium and nystatin treatment. Less than 7% of genes that exhibit a significant increase in messenger RNA expression are also required for optimal growth in four of the tested conditions. Our results validate the yeast gene-deletion collection as a valuable resource for functional genomics.

Gene disruption is a fundamental tool of the molecular geneticist and allows the consequence of loss of gene function to be determined. For organisms with facile genetic methods and known genome sequence, it is possible to systematically inactivate each gene^{1–8}. Here we present the construction and initial characterization of the nearly complete set (96% of all annotated ORFs) of gene-disruption mutants in the yeast *Saccharomyces cerevisiae*. This directed approach provides major advantages over classical random mutagenesis and screening. First, the mutant phenotype reflects a complete loss of function of the gene. Second, as a ‘reverse genetic’ approach, the previously laborious task of identifying the gene responsible for the mutant phenotype is accomplished beforehand. Moreover, in contrast to random mutagenesis, where genes often

elude detection even when a large number of mutants are screened, mutant ‘saturation’ of the genome is assured.

Deletion strategy

Each gene was precisely deleted from the start to stop codon (non-inclusive) and replaced by mitotic recombination with the *KanMX* deletion ‘cassette’ shown in Fig. 1 (ref. 9). The *KanMX* gene in each resulting mutant is flanked by two distinct 20-nucleotide sequences that serve as ‘molecular bar codes’ to uniquely identify each deletion mutant (see Methods for details of the design and construction of these sequence tags). Each deletion was verified by several polymerase chain reactions (PCRs), as described in Supplementary Information. In total, we deleted 5,916 genes (96.5% of total

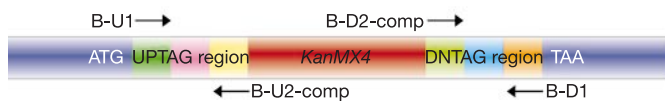


Figure 1 The *KanMX* deletion cassette module. The biotin-labelled, deletion-specific primers (B-U1, B-U2-comp, B-D1 and B-D2-comp; see Methods for structure) are used to amplify the unique UPTAG and DNTAG sequences from genomic preparations generated in the fitness-profiling studies.

attempted; see Methods for a complete tally). Some 18.7% (1,105) of the genes proved essential for growth on rich glucose medium (a list of essential genes is available in Supplementary Information). Only about half of these (57%) were previously known to be essential (M. Cherry, personal communication). Non-essential ORFs are more likely to encode a new protein (17% of the non-essential ORFs and 9% of the essential ORFs encode new proteins); essential genes are more likely to have homologues in other organisms (82% of the essential genes and 67% of the non-essential genes encode proteins that are similar to a protein in another organism), according to the Munich Information Center for Protein Sequences (MIPS) Comprehensive Yeast Genome Database (<http://mips.gsf.de/proj/yeast/CYGD/db>). As expected, few of the essential genes are duplicated within the yeast genome: 8.5% of the non-essential genes, but only 1% of the essential genes have a homologue in the yeast genome.

Whole-genome parallel analysis

The unique sequence tags linked to each gene deletion allow the strains to be analysed in parallel. In each experiment, a mixed culture containing every deletion mutant is grown, samples are collected at several times during growth, and the molecular bar-code tags are amplified from genomic DNA (Fig. 1). The abundance of each deletion strain is then determined by quantifying the associated molecular bar codes by hybridization to an oligonucleo-

tide array of the complementary bar-code sequences. The more important a gene is for growth, the more rapidly the sequence tags of the corresponding deletion strain diminish in the culture. Thus, all genes required for growth can be identified and ranked in order of their relative contribution to fitness in a single experiment. In the following sections we present results from such fitness profiling of nearly all (96%) of the non-essential yeast genes under several well-studied experimental conditions. Data from these experiments can be found on our website (<http://genomics.lbl.gov/YeastFitnessData>).

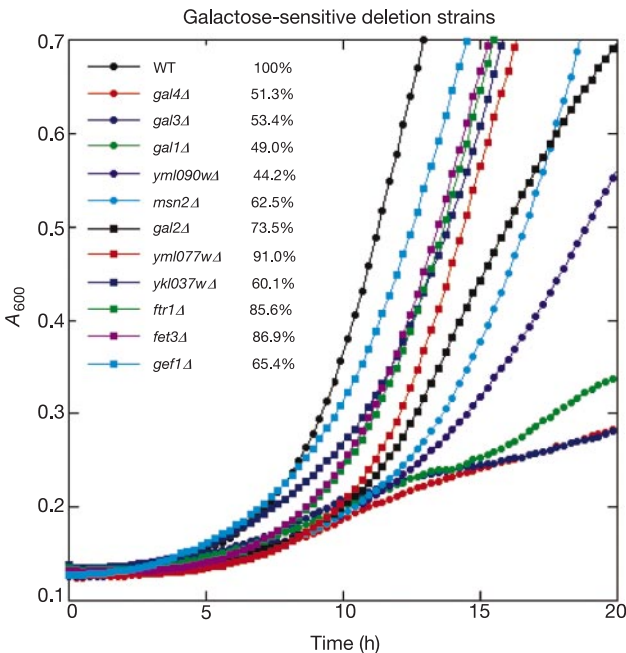


Figure 2 Growth of deletion strains exhibiting reduced fitness in galactose medium. Strains were grown overnight in YPD medium and diluted to 0.1 A_{600} in YPGal the next morning. Growth was in 24-well plates in a 340PC Spectramax spectrophotometer (Molecular Devices). Growth rates were calculated in the exponential phase of the growth curve. Percentage of wild-type (WT) growth is indicated in the legend.

Growth in rich medium

About 15% of all viable homozygous deletion strains exhibit a slow growth phenotype in rich medium at 30 °C (for a list of the strains and their functional categories see Supplementary Information Tables S1 and S2). Growth defects cover a continuous range from 12% to 90% of wild-type growth. Genes required for optimal growth under this condition are enriched in the functional categories of protein synthesis and cellular organization (according to the MIPS database¹⁰). Many of these genes encode ribosomal proteins (71.8% versus 59.4% in the whole genome) and proteins involved in mitochondrial function and respiration (28.2% versus 14.8% in the whole genome). Because these proteins are in high demand under such optimal conditions, it is not surprising that they are rate limiting for growth.

Growth in altered environmental conditions

Several adaptive responses to changes in the extracellular milieu have evolved in yeasts. To identify the genes involved in some of these responses, we surveyed all genes for a role in response to amino-acid availability, changes in carbon source, osmolarity/salinity, alkali, and challenge with the antifungal compound nystatin.

The yeast genes involved in amino-acid biosynthesis are well characterized¹¹. We were therefore surprised to discover that 13% of

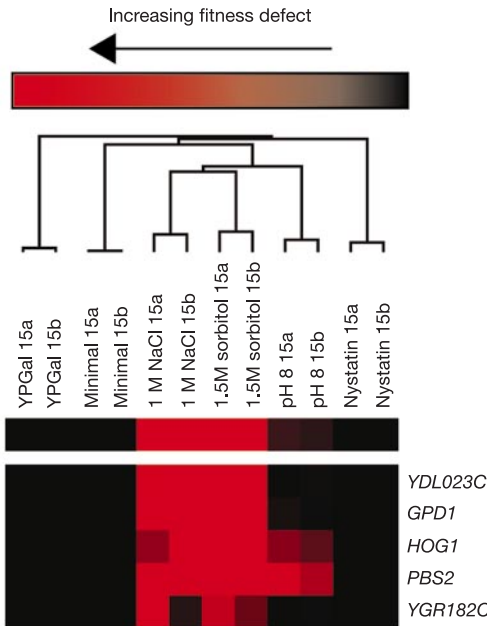


Figure 3 Clustering of genes required for growth in conditions of high osmolarity. Clustered data included fitness defect scores at 15 generations that were greater than 100 in replicate experiments (a, b). Genes were hierarchically clustered across all experiments using the program Cluster²² and viewed in TreeView (<http://rana.lbl.gov/EisenSoftware.htm>). The degree of fitness is represented by a colour bar, with bright red representing strains with the greatest fitness defect. Each row represents a single gene and its behaviour across all 12 experiments.

the genes required for optimal growth in minimal medium (lacking all but the required amino acids) are of unassigned function. When the pool was further grown in media that lacked only threonine, tryptophan or lysine, all genes known for biosynthesis of these amino acids (according to the Kyoto Encyclopedia of Genes and Genomes, <http://www.kegg.com>) were identified. In addition, a new gene—*YJL200c*—that shares similarity with known aconitate hydratases was identified as the component probably responsible for the second step in the lysine biosynthetic pathway (conversion from homocitrate to homo-*cis*-aconitate).

The use of galactose is one of the best-studied pathways in yeast, yet we identified ten genes not previously known to be required for optimal growth on this carbon source: *MSN2*, *FTR1*, *FET3*, *YDR290W*, *ATX1*, *YNL077W*, *YDR269C*, *GEF1*, *YML090w*,

YKL037W (the growth defect of *ykl037wΔ* is probably due to partial disruption of the 5' region of the neighbouring *UGP1* gene, which is required for galactose use). When particular deletion strains were tested individually, they exhibited 44–91% of the wild-type growth (Fig. 2). Thus, fitness profiling can discover genes involved even in previously well-studied pathways.

In wild-type cells, changes in extracellular solute concentration are monitored by two osmotic sensors that independently activate the HOG (high osmolarity glycerol) signal transduction cascade by phosphorylation of Pbs2 (a mitogen-activated protein kinase kinase, or MAPKK). Pbs2 activates Hog1 (a MAPK) that, in turn, leads to the production of Gpd1 (ref. 12). Gpd1 catalyses the rate-limiting step in glycerol production, the process that ultimately returns the cell to homeostasis. As expected, all three of these genes were required for growth in 1.5 M sorbitol and 1 M NaCl (Fig. 3). Three other deletion mutants—*ygr182cΔ*, *gsc1Δ* and *ydl023cΔ*—exhibited significantly reduced fitness in conditions of high osmolarity. Two of these genes were not previously known to be involved in this process. *YGR182c*, a gene expressed upon exposure to 1 M NaCl (ref. 13), clustered with the known responders to osmotic stress discussed above, implying similar function (Fig. 3). In contrast, the *gsc1Δ* mutant, in addition to its sensitivity to high osmolarity, exhibited decreased fitness in minimal and pH 8 media. *GCS1* encodes an ADP-ribosylation factor (ARF) GTPase-activating protein (GAP) protein required for secretion, the absence of which may disable the function of membrane proteins required in these conditions¹⁴. The third unknown ORF required for optimal growth at high osmolarity, *YDL023*, overlaps *GPD1*, suggesting that the phenotype is due to the disruption of the *GPD1* gene and not related to the potential function of *YDL023*.

In addition to causing osmotic stress, 1 M NaCl disturbs ion homeostasis and is ultimately toxic to yeast cells. In response to this insult, cells increase the activity of several of the P-type ATPases, which remove Na⁺ from the cytoplasm through the calcium–calcineurin pathway. We discovered that the calcineurin-related genes *RCN1* and *CNB1* and the protein kinase *HAL5* are critical for growth under ionic stress. Strains deleted for these genes are also sensitive to conditions of high pH, suggesting commonality in the cellular response to these two conditions. Salt-specific targets include the calcineurin-dependent transcription factor Crz1, the ion-transport-related proteins Npr1 and Sat4, components of the Rim1 pathway (Rim101, Rim13), and Sro7. In total, we identified 62 salt-hypersensitive mutants, 47 of which were previously unknown despite two previous genome-wide efforts to identify such genes^{13,15}. It should be noted that deletions of the genes encoding the major ATPases involved in Na⁺ efflux (*ENA1*, *ENA2* and *ENA5*) are not in the yeast knockout collection because their duplicated nature prevented the automated selection of unique primers for making systematic deletions.

In contrast to the pathways regulating growth in response to salt, the cellular response to alkali has not been extensively studied. We identified 128 alkali-hypersensitive mutants, 100 of which are specific to alkali stress, indicating that the cellular response to high external pH is distinct from ionic stress, despite several shared components. Inspection of the genes required for survival in alkaline conditions suggests that proper cell wall maintenance and vesicle transport are required for optimal growth at high pH. These include: components of the Bck1–Slr2 cell wall integrity pathway; members of the Hog1 pathway (Fig. 3), and several members of clathrin-associated protein (AP) complexes. The role of these clathrin-associated proteins in vesicle transport suggests that this process is important for yeast adaptation to high external pH.

Nystatin, one of the oldest and most effective antifungal drugs, causes cell death by binding to membrane ergosterol and creating pores in the plasma membrane. Two of the deletion strains most sensitive to nystatin, *myo5Δ* and *bro1Δ*, are required for cell wall structure and integrity. Consistent with the concept that nystatin

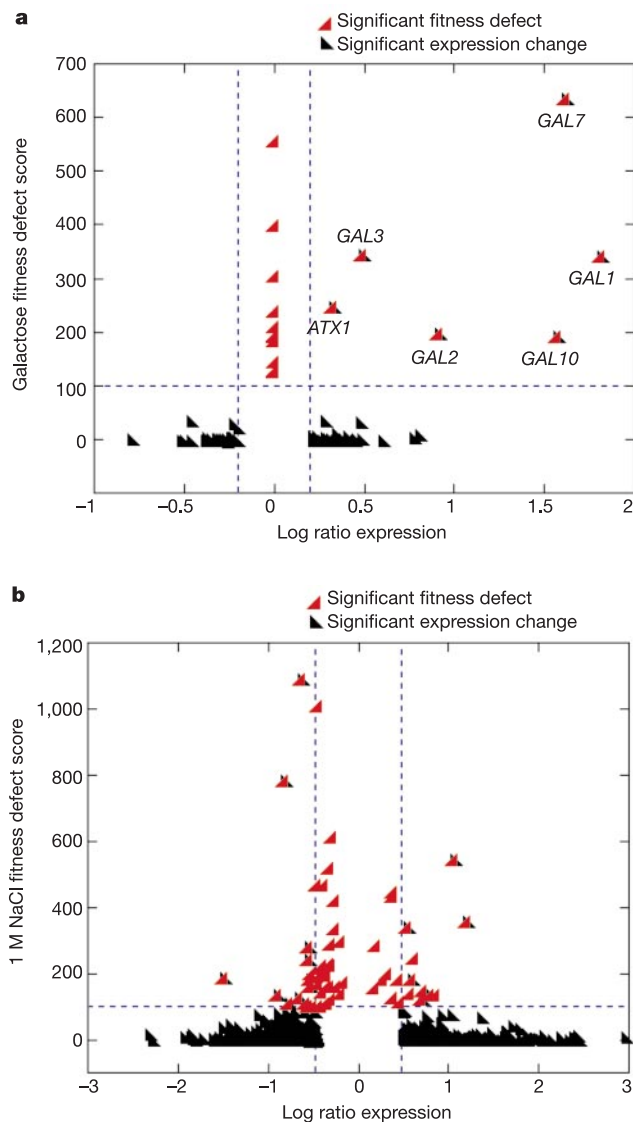


Figure 4 Comparison of expression and fitness profiling data. For clarity, only those genes designated as sensitive by the fitness defect score were plotted. Red triangles represent genes with significant fitness defect scores (above the dashed line) plotted as a function of their corresponding values for log ratio expression: $\log(\text{condition expression}/\text{reference expression})$. Black triangles represent genes with significant log ratio expression (outside the two vertical dashed lines) plotted with their corresponding fitness defect scores. The values of the fitness defects plotted are the minimum score from two experiments. **a**, Galactose. The six points that overlap are significant in both experiments (*GAL1*, *GAL2*, *GAL3*, *GAL7*, *GAL10* and *ATX1*). **b**, 1 M NaCl.

further compromises deletions that are defective in different aspects of membrane integrity, seven of the genes required for optimal growth in 10 μ M nystatin encode either integral or peripheral membrane proteins (*VPS8*, *VPS24*, *VPS28*, *BSD2*, *GIT1*, *MAL11* and *VPS24*). Several other genes required for nystatin resistance are involved in intracellular transport (*STP22*, *YDL100C*, *SRN2*, *SIP3*, *SNF7* and *VPS30*). Eleven genes required for maximal growth in the presence of nystatin are of unknown function, indicating that we still have much to learn about the cellular effects of this compound.

Comparison of fitness and expression profiling

Because both expression profiling and fitness profiling interrogate the whole genome simultaneously, we asked whether a relationship exists between the change in mRNA expression of a gene and its requirement for growth in the same condition. For this comparison, we used previously collected data^{15,16} because strains with the same genetic background as the deletion strains were used in those studies, and expression changes were monitored in four of the same conditions (1 M NaCl, 1.5 M sorbitol, pH 8 and galactose). Our hypothesis was this: if a gene exhibits a significant increase in expression in a given condition, then it should also be required for optimal growth in that condition. We found that in galactose, less than 7% of the genes that exhibited a significant increase in mRNA expression also exhibited a significant decrease in fitness. In the case of pH 8, 1 M NaCl and 1.5 M sorbitol, 3.0%, 0.88% and 0.34%, respectively, of the genes that exhibited a significant increase in mRNA expression also exhibited a significant decrease in fitness (see Supplementary Information). Moreover, many of the genes that exhibited a significant fitness defect did not exhibit a significant change in expression (Fig. 4, see also Supplementary Information). The fact that such a small percentage of the genes that exhibit a significant increase in expression also exhibit a significant fitness defect was unexpected and warrants closer inspection.

Cell shape and size

To identify genes involved in specifying cell shape and size, we visually screened 4,401 of the homozygous diploid deletion mutants by differential interference contrast (DIC) microscopy of fixed cells.

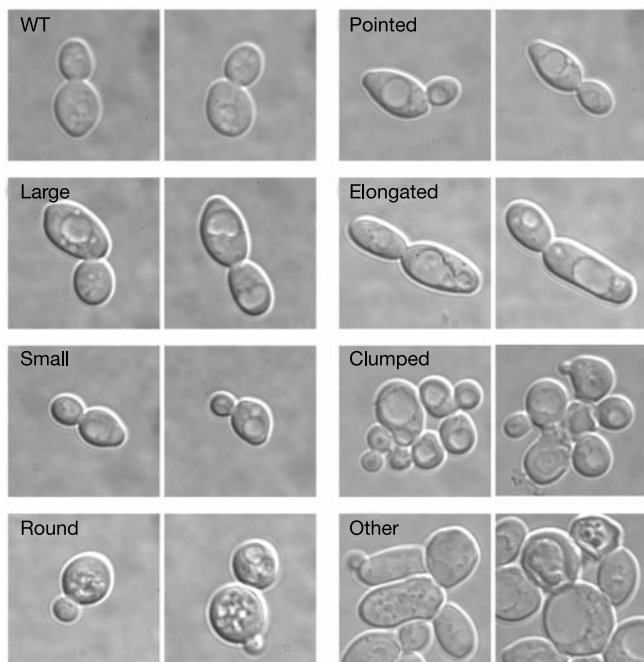


Figure 5 The seven phenotypic categories of deletion mutant morphologies. WT, wild type.

We identified 673 (~15%) deletion strains exhibiting slight to strong morphological alterations from the normal ellipsoid cell shape of wild-type diploid cells. The deletion mutant morphologies were grouped into seven classes: 'elongated', 'round', 'small', 'large', 'pointed', 'clumped' and 'other' (Fig. 5). Mutants with more than three kinds of morphological phenotypes were classified as 'other'. Using the MIPS functional classification system, we found that clumped and elongated strains were enriched for mutations in genes for cell growth, cell division and DNA synthesis (28.9% and 18.9% of clumped and elongated strains, respectively, versus 10.6% for the whole genome). In addition, round strains were enriched for mutations in protein synthesis genes (14.9% versus 3.1% for the whole genome). These latter mutants are also defective in bud site selection, consistent with the hypothesis that apical growth is important for bud site selection¹⁷. A summary of the number of different deletion mutants in each phenotypic category is presented in Supplementary Information along with a detailed list of these strains.

Discussion

The sequence tags that uniquely identify each gene disruption enable functional analysis of the deletion collection on an unprecedented scale. Using this method, we scored the fitness of each homozygous deletion strain (and therefore the requirement for its gene product) under six different conditions. These results confirmed genes known to be required for the different stress conditions, but, more importantly, revealed new genes involved in these processes. Although we did not, to our knowledge, miss any of the genes involved in these pathways, we do expect a small percentage of false negatives in cases where the sequence tags of a strain hybridize poorly to the oligonucleotide array. In some cases, fitness profiling allowed the identification of the gene in a gene pair or family primarily required for a particular process. For example, the *gpd1Δ*, but not the *gpd2Δ*, deletion mutant exhibited reduced fitness in 1.5 M sorbitol. That we uncovered previously unknown genes even in such well-studied pathways as galactose use and amino-acid biosynthesis suggests that we have achieved a higher level of saturation genetics, avoiding the biases known to exist using conventional screens^{3,18}.

We observed little overlap of genes identified both as significant by fitness profiling and as significantly upregulated by gene expression profiling in conditions of 1 M NaCl, 1.5 M sorbitol, pH 8 and galactose. It is easy to imagine why some genes required for growth under a particular condition do not exhibit a change in expression in that condition, because the response to the change in condition may operate post-transcriptionally. The converse situation—a gene that exhibits a significant increase in expression but is not required for growth—is quite surprising, and more difficult to comprehend. It is possible that under stress conditions, multiple gene products are expressed, only a small fraction of which are essential for adaptation to the specific condition in question. Some of these differences might also be ascribed to the highly duplicated nature of the yeast genome. Whatever the cause, fitness profiling may help to identify the subset of genes identified in expression profiling that are required to be expressed to adapt to the condition in question. □

Methods

Deletion strains, primer choice and synthesis

For details of deletion strain construction and primer choice and synthesis, see Supplementary Information. For strain availability, see our website (<http://www-deletion.stanford.edu>).

Selection of genes to be deleted

The initial annotated ORF list obtained from the *Saccharomyces* genome database (SGD, <http://genome-www.stanford.edu/Saccharomyces>) included 6,227 unique ORFs, but was pared down to 6,131 ORFs after removal of ORFs that are not unique owing to gene duplication or regions of high sequence similarity. Of these, we generated four yeast gene

knockout (YKO) collections: 4,815 *MATa* and 4,803 *MATα* haploid deletion mutants (independently generated) deleted for non-essential genes, 4,757 homozygous diploid deletion mutants missing non-essential genes, and 5,916 heterozygous diploids (including essential and non-essential genes). We failed to delete 215 genes for unknown reasons; about 62% of these are questionable ORFs that have no known biological function. The list of ORFs not deleted in the YKO collection can be found in Supplementary Information.

Media and growth conditions

YPD (yeast extract, peptone, dextrose) and synthetic minimal media were prepared as described^{19,20}. Minimal drop-in medium included histidine, uracil and leucine, which are required for growth of the deletion strains. We added 1 M NaCl and 1.5 M sorbitol as supplements to YPD before autoclaving. YPGal medium is equivalent to YPD medium except that 2% galactose is substituted for 2% dextrose. We made pH 8 medium by titrating YPD with 1 M Tris-HCl buffer at pH 9.6 ($\sim 10 \text{ ml l}^{-1}$).

Deletion pool construction and growth

Pools of the deletion mutants were prepared as follows: batches of 96 deletion strains were applied in patches to YPD plates and grown for 3 days at 30 °C. Approximately five absorbance units at 600 nm (A_{600}) of cells of each strain were collected from solid medium with wooden toothpicks and added to 25 ml of YPD plus 15% glycerol. The subpools were stored in 1-ml aliquots at -80°C . To construct the whole genome pool, subpools were thawed and mixed together such that the average A_{600} per strain was equivalent and aliquots were stored at -80°C . In each experiment, $\sim 6 \times 10^6$ cells from a freshly thawed pool aliquot of homozygous deletion mutants ($\sim 10^3$ cells per strain per culture) were inoculated in YPD and grown overnight to allow about ten generations of recovery from storage at -80°C . Cells were then diluted into 50 ml of the appropriate pre-warmed fresh media and grown at 30 °C with shaking at $\sim 250\text{--}300$ r.p.m. in 250-ml flasks. To minimize sampling errors while maintaining logarithmic growth, cultures were batch diluted as necessary to not less than $\sim 10^3$ cells per strain. We collected 2 A_{600} of cells from the cultures at 5 and 15 generations after the recovery period and froze them at -20°C for subsequent preparation of genomic DNA.

Genomic DNA preparation, PCR and chip hybridization

DNA from 2 A_{600} of cells was prepared after lysing the cells either with glass beads²¹ or enzymatically using a Qiagen DNeasy kit. The UPTAG and DNTAG molecular bar codes were amplified from $\sim 0.2 \mu\text{g}$ of genomic DNA in two separate reactions. The UPTAG amplification used primers B-U1 (5'-biotin-GATGTCCACGAGGTCTCT) and B-U2-comp (5'-biotin-GTCGACCTGCAGCGTACG); the DNTAG amplification used B-D1 (5'-biotin-CGGTGTCTCGTCTCGTAG) and B-D2-comp (5'-biotin-CGAGCTCGAATTCATCG) (Fig. 1). Amplified UPTAG and DNTAG sequences were combined and used to probe high-density oligonucleotide arrays (Affymetrix Tag3 array) in 150 μl of 1X hybridization buffer (100 mM MES, 1 M Na^+ , 20 mM EDTA, 0.01% Tween 20 and 1X Denhardt's solution) containing 1 μM of U1, U2, D1 and D2 oligonucleotides and their complements, and 0.6 fM of B213 control oligonucleotide. Samples were boiled for 2 min, chilled on ice for 2 min, and hybridized at 42 °C for 16 h. Washing, staining and scanning were performed as previously described².

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