Supplemental Materials

Molecular Biology of the Cell

French et al.
| Image Overview | Pipeline Step | Description |
|----------------|--------------|-------------|
| ![Image](image1.png) | Acquisition | Plates were arrayed two per scanner, and images were captured using the Linux bash command `scanimage` using SANE drivers. Acquisition occurred as parallel processes for each scanner. |
| ![Image](image2.png) | Pre-Processing | Images were analyzed using our supplied in-house macro/pipeline. Plates were separated, automatically rotated, and converted to 8-bit greyscale. |
| ![Image](image3.png) | Feature Identification | Thresholded images were used to find colony perimeters, and these selections were used to capture colony sizes across each plate in the image stack (timelapse). |
Colony Analysis

Original greyscale plates are background subtracted to remove media contributions to pixel density. Regions of interest (ROIs) from perimeter calculations are applied to original greyscale image stack, and integrated densities are returned for each colony in the timelapse.

Well Alignment

A grid system was aligned to the colonies, calculated by their pixel density across rows, and columns. Non-colonies such as bubbles are removed in this step.

Growth Rate Calculations

The initial inoculum is first subtracted from the growth curves, then curves are fit to the Gompertz model of growth. The maximum growth rate (\( \mu \)) coefficient is extracted from each model.

Endpoint Biomass

Endpoint biomass is retrieved from the final timepoint in the growth curves.

Normalization

Data are normalized using our supplied R functions.
Hits are detected using the system of (CITE), looking for sensitivity based on comparing the chemical-treated deletions to the product of chemical-only and knockout-only values.

**Figure S1.** Experimental workflow with example images. The workflow used in this experiment is illustrated above. It begins with scanning images kinetically, with 2 plates per scanner. Plate are
automatically rotated, and cropped by our analysis software. Each individual plate is converted to an 8-bit greyscale image, and the Otsu algorithm was used to generate a threshold mask. These masks were then used to identify the maximum colony margins. With the colony margins identified, a 50 pixel rolling ball background subtraction was performed on the original 8-bit greyscale image stacks, and the regions of interest calculated from the masks applied to the colonies. Integrated densities were acquired for each timepoint, and a grid system was calculated based on the additive colony density across rows and columns, in a similar manner as Wagih and Parts (Wagih and Parts, 2014). Growth curves are compiled, and the maximum growth rate calculated as the $\mu$ coefficient from a fit Gompertz model of growth. Endpoint biomass and growth rate measures are normalized using our R function (provided in Supplementary Data, and further explained in methods). Hit are determined by the deviation from the expected value, calculated via the multiplicative rule. This is further described in the materials and methods.

**Figure S2.** Various screening quality control measures. Shown in (a) is the linear relationship between colony forming units (CFU) and raw integrated density values as acquired in ImageJ. Individual colonies were cut out using a scalpel, vortexed briefly in LB broth medium, then finally serial dilutions were plated out on LB agar. This illustrates the value of the integrated density parameter in biomass estimation, and validates the use of the parameter in growth rate calculations. In
(b), the reproducibility of the normalized total biomass accumulation is shown. An example 1536-well plate is shown in (c), and an individual colony raised into 3D based on its pixel grey value is shown in (d). The pixel grey value is what the integrated density is calculated from, and integrated density is essentially a measure of total colony biomass.
**Figure S3.** Solid media MIC examples inoculated using the Singer RoToR with the same inoculum as the chemical genomics experiments. This is a crucial step, as it maintains consistency in the immense inoculum of solid medium arraying. Shown in (a) is an MIC curve from large solid medium plugs (about 1.2 mL volumes), alongside (b) small plugs (about 450 μL volumes). This greatly decreases the amount of drug needed to do solid medium MIC determinations. The larger plugs are more ideal for 1536-density plates, whereas the small plugs shown in (b) work well with 6144-density plates.

**Figure S4.** Network interaction map for cell wall drugs assayed in this study. As in Figure 7, the black edges represent endpoint biomass interactions, while green edges represent a slow growth interaction. Interactions are defined by the multiplicative approach explained in the text body. The layout clearly shows nodes shared between D-cycloserine, ampicillin, and fosfomycin. D-cycloserine and fosfomycin have cytoplasmic targets, which is likely why there are more shared interactions between these two drugs, than with ampicillin. Many shared nodes are discussed in the body of the manuscript, with lists of hits provided in supplementary data.
Figure S5. Network map outlining interactions between protein translation inhibitors chloramphenicol, erythromycin, tetracycline, spectinomycin, and streptomycin. Interactions are defined using the multiplicative approach discussed in the main body of the text. The edges are colored in the same manner as Figure 7; black edges represent sick or lethal interactions, and green edges represent slow growth interactions. Shared nodes are discussed in the body of the text, and annotated with the function of their gene product.
Figure S6. Interaction map illustrating the interactions between DNA replication inhibitors nalidixic acid, ciprofloxacin, and norfloxacin. As with the interaction map shown in Figure 7, black edges are representative of a low biomass accumulation ‘endpoint’ phenotype, while green edges represent slow growth phenotypes. Interactions are calculated through the deviation from the expected value via the multiplicative approach explained in the body of the manuscript. Shared nodes are discussed in the body of the text.
Figure S7. Network interaction map combining both endpoint biomass and growth rate interactions, and clustered using a Markov clustering algorithm. A relatively low inflation was used to get a coarse representation of genetic clusters based on genetic interactions. This resulted in 5 major clusters, which were exported to EcoCyc to acquire GO terms in batch. Across the various clusters, interactions are enriched for key cellular processes, with transport, redox processes, and antibiotic response being essentially conserved across all clusters. The top GO terms with respect to frequency of occurrence are depicted in the legend.
**Figure S8.** Kinetic acquisition setup for agar plates. We utilize a computer controlled power source that regulates power to the transmissive plate scanners, in order to control the temperature of the scanner lamp. Shown in (a) are temperature profiles for scanners in a 37°C incubator when the lamp power is not controlled (red), and when power to the scanner lamp is regulated using our setup (blue). Not regulating the lamp can result in plates where media is immensely thin and dried out. This occurs due to the lamp staying on for about 15 minutes post-scanning. In (b) we outline the schematics for our kinetic acquisition setup, including the network-controlled power bar that regulates the scanner lamps shown in (a). The resulting plate transition timelapse is shown in (c), and growth curves for a 1536-well plate are shown in (d). Curves are presented as raw integrated density, with the initial inoculum background subtracted. Highlighted in red, is the approximate zone where the log-transformed data is linear. This is the area most essential to calculating growth rate.