Acquired Stress Resistance Assay

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Abstract

A simple 96-well plate assay for stress resistance across a range of stress doses. Originally designed for yeast, but easily modifiable for any colony-forming microbe.

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Guidelines

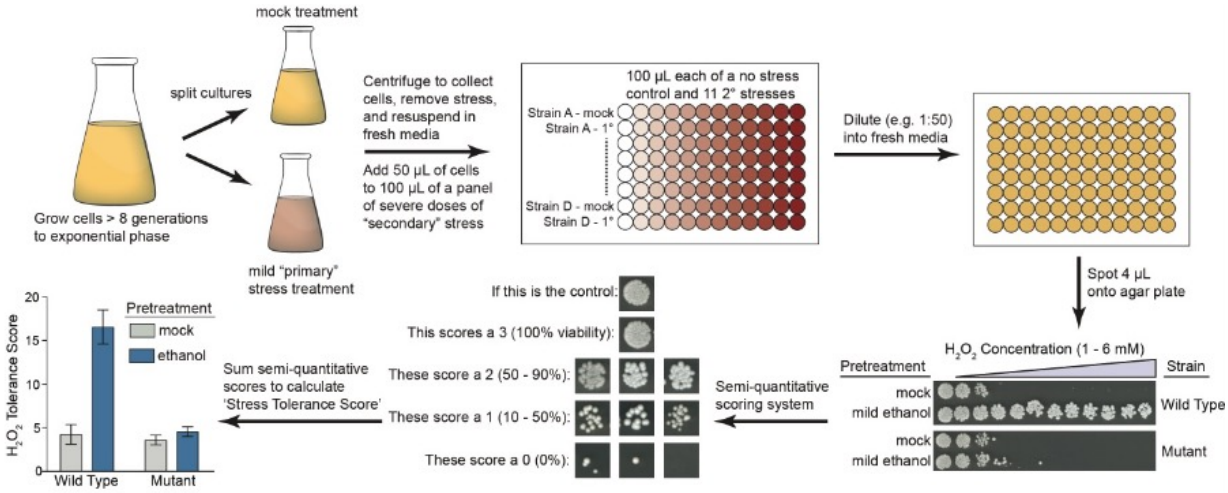
Stress assays are very sensitive to differences in growth conditions. In our hands, we have noticed that relatively small differences in temperature, shaking speed (aeration), and media composition can affect cellular stress resistance. To test media consistency when we receive a new batch of media, we perform control experiments on cells grown with both the old and new media lots. To maintain consistency across shaker platforms, we use a digital tachometer to ensure identical shaking speed, and we measure the temperature of a control flask of water every morning.

Timing is also a very important consideration. We use multi-channel pipettors or pinners and work quickly, especially when transferring cells from the secondary stress to fresh media. We process a maximum of two 96-well plates at a time to ensure that viability differences across strains are not due to differences in exposure time to the secondary stress. This was determined in our lab empirically for our yeast strains using technical replicates across multiple plates, and we would recommend this procedure when adopting this protocol for new organisms or stress exposure times.

Protocol

Schematic of the assay

Step 1.



Obtain exponentially growing yeast cells

Step 2.

- Starting with a saturated culture, sub-culture and grow cells at 30°C with 270 rpm orbital shaking at least 8 generations to early-to-mid exponential phase (OD_{600} 0.3 - 0.6 on a Unico 1100RS Spectrophotometer). Growing cells at least 8 generations is critical for resetting their epigenetic 'memory' of stationary phase, which transiently increases stress resistance (see DOI: 10.1534/genetics.112.143016).
- For convenience, we generally grow cells overnight for 12-16 hours (yeast doubling time is 90 minutes in rich medium). Standard volumes are 6 ml (20-mm tubes) or 30 ml (125-ml flasks).

Prepare severe (secondary) panel of stresses

Step 3.

- The doses should be prepared at a 1.5X concentration, since cells will be diluted 1:2 (50 µl cells into 100 µl stress media).
- For each 96-well plate to be assayed, pipette 1 ml of each of the 11 'severe' stress doses into a single well of a deep-well 96-well plate (lengthwise). One lane (either the first or last) should serve as a no stress (media alone) control.
- Use a multi-channel pipettor to pipet 100 µl of each dose into a 96-well microtiter plate.
- Note: for stresses that evaporate or decay (e.g. H_2O_2 or ethanol), prepare stress plates 30 minutes into the 'primary' treatment (Step 3).

- Secondary doses should be empirically determined to cover the range of tolerance across strains. As a starting point, common secondary stress doses in our lab are: NaCl (0.75 M to 3.25 M in 0.25 M increments); H_2O_2 (0.5 mM to 5.5 mM in 0.5 mM increments); EtOH (12.5% (v/v) to 17.5% in 0.5% increments); Heat (37°C - 57°C using the gradient function of a thermocycler).

Mild (primary) stress treatment

Step 4.

- Primary stress doses are generally prepared as a 2X stock.

- Split cultures for primary and mock treatment: add 2.5 ml of the culture to two 20-mm test tubes.
- Add 2.5 ml of primary treatment (e.g. 0.4 mM H_2O_2) to one tube and 2.5 ml of mock treatment (equivalent volume of water/carrier solution) to the other tube.
- Incubate at 30°C with 270 rpm shaking in orbital shaker. The amount of time necessary to induce optimal acquired stress resistance should be determined empirically, but 1 hour is the average for most stresses for yeast (see DOI:10.1091/mbc.Z08-00-0011).

- We define mild stress as a concentration that provokes an expression response to stress, but where cells still maintain >95% viability. This may need to be determined empirically, but as a starting point common primary stress doses in our lab are: NaCl (0.7 M); H_2O_2 (0.4 mM); EtOH (5% (v/v)); Heat (25°C - 37°C, or 30°C - 42°C).

Severe (secondary) stress treatment

Step 5.

- Following primary incubation, measure the OD_{600} of the mock and primary treated cells and record.
- Collect the cells by gentle centrifugation (3 min at 1500 rcf), decant media, and resuspend in fresh media to an OD_{600} of 0.6 (e.g. if OD_{600} of cells was 0.3, resuspend cells in 2.5 ml for a final OD_{600} of 0.6).
- Pour each cell sample into a sterile boat and use a multichannel pipettor to add 50 µl of each sample to the secondary stress plate (lengthwise) (150 µl total volume per well).
- Place a breathable rayon seal (VWR Cat# 60941-084) over the 96-well secondary stress plate.
- Incubate at 30°C with 800 rpm shaking for 2 hours in a microplate shaker (VWR Symphony Cat# 12620-930) (time can be changed empirically).

Dilute cells onto large agar plate

Step 6.

- Dilute cells 1:50 (e.g. 4 µl into 196 µl) and spot onto a large agar plate (rich medium).
  The dilution for the control spot should be nearly a lawn, and may need to be determined empirically.
- Plates should be dry enough that the spots do not run together. Be careful not to disrupt the plate until the spots have soaked into the plate.
- Incubate plates at 30°C for 24-48 hours before scoring.

Semi-quantitative scoring

Step 7.

- Viability can be scored quantitatively (via flow cytometry or colony counts) or semi-quantitatively as described here.
- We use a 4 point scale compared to the control spot: 3 pt = 100% viability, 2 pt = 50 - 90%, 1 pt = 10 - 50%, 0 pt = 0% (3 or less individual colonies).
- An overall tolerance score can be calculated as the sum of scores over the 11 doses of evere stress.