Yeast growth and viability assay

-Three biological yeast samples with the ALD2 WT, KO, and ALD2*

-Each biological condition will be under 0% (positive control), 12.5%, 25% ethanol treatments in the yeast media with two technical replicates each. (18 replicates)

-Set up negative controls that have no yeast but have 0% (positive control), 12.5%, 25% ethanol with two technical replicates. (6 more replicates)

Yeast culture

Repeat this procedure for each gene treatment in the culture plates.

1. Makeup 200 mL of YPD broth by dissolving 2 g yeast extract, 4 g peptone, and 4 g dextrose in 200 mL water.
2. To 100 mL of YPD broth, add 2 g agar and dissolve well.
3. Autoclave the YPD broth and YPD agar for 30 min.
4. Clean the work area for yeast culture.
5. Pour the YPD agar into plates, add the required ethanol in % w/v and cool to set.
6. Scrape a single colony from the culture plate that contains the differing gene samples and inoculate the three YPD broth with it, and again add required ethanol in % w/v.
7. Incubate the YPD broth at 30°C overnight. (Yeo, 2020)

Spectrophotometry to determine yeast numbers

1. Prepare 1 mL each of undiluted and diluted samples of the yeast culture. Use YPD broth to dilute the yeast culture.
2. Set up the spectrophotometer. Set wavelength to 600 nm.
3. Blank the spectrophotometer with a cuvette containing 1 mL of YPD media (without yeast cells). Make sure that the absorbance reading at 600 nm is zero. Remove this cuvette from the spectrophotometer.
4. Transfer the first culture sample to a new cuvette. Measure the OD_{600} of the sample by looking at its absorbance at 600 nm. Repeat for all other samples. (Yeo, 2020)

Spot plate assay for yeast viability

1. Harvest ~73 million yeast cells in mid-log phase by centrifuging the culture at 3900 g for 3 min.
2. Remove the supernatant and resuspend the cells in 20 mL pre-warmed YPD broth. Mix well.
3. Add 10 mL of culture to each of two tubes.

4. To one tube, add H_2O_2 to a final concentration of 4 mM. (Stock concentration is 1.96 M, so 20.4 µL of the stock gives a final concentration of 4 mM in a 10 mL final volume.)
5. Incubate both tubes at 30°C for 30 min.
6. During the incubation time, prepare two YPD agar plate for spot plate, one containing 3 rows of dilutions with the rows being 0%,12.5% and 25% ethanol respectively. The second plate will be identical but with the hydrogen peroxide added. On a sheet of paper, make an alignment grid and mark the target positions for the culture dilutions. Place an orientation mark at one point along the circumference. Make a corresponding mark on the bottom half of the YPD plate, which will line up with the orientation mark on your alignment grid.
7. After the 30 min incubation, take 1 mL from the untreated and treated cultures and centrifuge at 12,000 g for 2 min.
7. Remove the supernatant and resuspend each cell pellet in 1 mL sterile water.
8. Set up a series of five 1:10 dilutions for each culture sample. In the first tube, add 200 µL of undiluted cells. In the next five tubes, add 180 µL of sterile water. Take 20 µL of the undiluted cells and add it to the next tube containing 180 µL of water. Pipette to mix, then take 20 µL of this dilution and add it to the next tube. Repeat to finish the dilution series. Do the same for both untreated and treated cultures for both plates.
9. Beginning with the last dilution in the series, spot 5 µL from each tube onto the YPD plate. Mix each dilution before spotting because cells may have settled in the suspension.
10. Let the spots dry on the plate. Invert the plate, and label around the bottom rim of the dish. Incubate at 30°C for 48 hrs until the colonies are large enough to count.
11. Use the spots where you can count individual colonies to calculate the concentration of live cells in the untreated and treated cultures, taking into account the volume spotted and the dilution factor.(Yeo, 2020)

Analysis: Visual and the subsequent mathematical analysis will provide information on the growth rate and viability of the yeast cells. This can then be visualised using programs such as GraphPad Prism or Excel and statistical significance can be evaluated using an unpaired *t*-test (Tran & Green, 2019).

References for protocol and yeast video:

Tran, K., & Green, E. M. (2019). Assessing Yeast Cell Survival Following Hydrogen Peroxide Exposure. *Bio-protocol, 9*(2), e3149. doi:10.21769/BioProtoc.3149

Yeo, G. (2020). *Yeast Growth and Viability (Week 3): Protocol and notes* Retrieved from https://au-mynotebook.labarchives.com/