Last Modified: 180924 11:07
By Robert Newberry

Selection Experiments

Goal:
Differentiate a-synuclein variants by their toxicity in yeast exposed to a chemical perturbation

Before you came to class:
A wild-type yeast strain (S. cerevisiae, W303 background) was transformed with a plasmid library expressing variants of a-synuclein-GFP under control of the GAL promoter. The library contains all possible point mutants of a-synuclein. A glycerol stock was thawed and brought up in SCD-Ura for 24 hours before inoculation into SCR-Ura overnight. The SCR-Ura culture was then diluted the following morning. You will receive the culture after approximately 10 hours of growth, at which point the cells should be in the log phase.

Procedure:
Monday, during class

1) Determine the appropriate compound concentration and starting yeast density for selection. Compound concentration should impose a growth defect, and the starting yeast culture density should be such that the final OD after 12h of growth is between 1.0 and 3.0. Use your pilot data to guide your choices.
2) Prepare 250 mL of SCR-Ura with 1% galactose and the appropriate concentration of your compound.

Selection Schedule	Replicate 1	Replicate 2
Monday, 7:30pm	Collect time=0h samples Induce expression	
Tuesday, 8:30am	Collect time =12h samples Dilute culture	
Tuesday, 7:30pm	Collect time=24h samples	Collect time=0h samples Induce expression
Wednesday, 8:30am		Collect time=12h samples Dilute culture
Wednesday, 7:30pm		Collect time=24h samples

Inducing expression:

1) Determine the OD of the uninduced starter yeast culture.
2) Determine what volume of starter culture is necessary to inoculate a 50 mL culture to the appropriate starting OD.
3) Transfer that volume of starter culture to a conical vial.
4) Pellet cells at 2500g for 10 minutes.
5) Isolate the pellet by aspirating off the media.
6) Resuspend the pelleted cells in 50 mL of your prepared media.
7) Determine the OD of the resulting culture.
8) Adjust the OD as necessary.
9) Return the flask to shaking at 30C, 200 rpm.

Collecting samples for miniprep:

1) Determine the OD of the yeast culture.
2) Collect **two** samples of your culture, each containing 25 ODs. 1 OD is 1 mL of culture having an OD of 1. So, if your culture has an OD of 2, you need 12.5 mL for each aliquot. Transfer to a conical vial.
3) Pellet the cells for 10 minutes at 2500g.
4) Isolate the pellet by aspirating off the media.
5) Resuspend the pelleted cells in 1 mL of water and transfer to a microfuge tube.
6) Pellet the cells for 1 min at 12000g.
7) Isolate the pellet by aspirating off the media.
8) Label your tubes with your team name, the date, the replicate number, and the time point.
9) Place on ice until all groups are done.
10) Transfer samples to -80C storage until miniprep.

Diluting yeast culture:

1) Using the cells left over after collecting your miniprep sample, prepare a new 50 mL culture in a new flask at the same starting OD as before. Make sure to use the appropriate media.