Yeast Farming for Microarrays

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This protocol describes a typical perturbation microarray experiment in yeast. It is meant to convey the details that are important for all such experiments and can be used as a model for other kinds of perturbations. The important points are that the time course be done in a systematic, re-producible manner, with as few variables differing as possible. This includes media preparation and pre-cultures. Also, rapid harvesting by filtration to avoid non-specific gene expression responses.

Day 1: Preparation

Inoculate several overnight cultures (*to ensure you'll have one near OD_{600} = 5.0 on day 2*) in 500 ml flasks with 100 ml YEPD, grow at 30 °C.

Make media for Day 2:

1 L YEPD in a 2.8 L Fernback flask
(*I use flasks with baffles for better aeration*):

- 20 g Peptone
- 10 g Yeast extract
- 960 ml H2O
- Autoclave to sterilize
- Add 40 ml 50% glucose to media after autoclaving

500 ml YEPD + 1.25 M KCl in any container:

- 10 g Peptone
- 5 g Yeast extract
- 46.6 g KCl
- add H2O for 480 mls total volume
- Autoclave to sterilize
- Add 20 ml 50% glucose after autoclaving

Place autoclaved media into 30 °C incubator to pre-warm it.

> **Note about media:** I've noticed batch to batch variation in Peptone and/or Yeast extract composition, so I take a bottle of dry Peptone and Yeast extract for my own use with microarray work. This way, for a related set of experiments I always use the same media. With different batches of media, yeast show varied expression of iron and copper-regulated genes, so presumably the metal composition of the media is variable. This is most important if you use a "master zero sample" or a "master pool" to hybridize multiple samples against.

Day 2: Harvest

Check 1:10 dilutions of the overnight cultures for OD_{600}
(note that spectrophotometers can vary for OD_{600} readings)

When OD_{600} = 5.0, take 20 mls of the overnight and inoculate the 1 L YEPD. Take a new OD_{600} of this new culture and calculate how much more of the overnight culture to add to obtain OD_{600} = 0.15 using the formula below. Discard unused overnight cultures

(3 / X = total mls of overnight to add, where X is the OD_{600} after adding the 20 mls.)

> **Note about growing yeast:** The OD_{600} readings I grow yeast to were chosen for convenience. You might choose different cell densities for your experiments, but be consistent day to day for whatever you use, that way you can compare gene expression patterns without worrying about culture condition artifacts.

Incubate the 1 L culture at 30 °C, on a 200 RPM shaker.

Measure OD_{600} periodically.

Prepare filter apparatus, shown below. (*We use a 90 mm, 1L glass Fisher unit: cat. #K953755-0090 with 0.45 µm Nitrocellulose filters*), label 50 ml disposable plastic tubes for use later, and have liquid Nitrogen ready.

When OD_{600} = 0.6 (*usually after 3-4 hours*), begin the time series.

Take a 250 ml sample for the "zero" time by pouring into a 0.5 L graduated cylinder. Dump in the 0.5 L of KCl media and start a timer counting up.

Collect the zero sample by using the filter apparatus. After all of the media has been removed, disassemble the unit and roll the filter up and place in a labeled 50 ml conical tube. Put tube into liquid Nitrogen. Prepare filter unit for collecting another sample.

After 10 minutes, take a 250 ml "10min." sample.

Harvest samples for 20min, 30min, and 40min.

Freeze sample tubes in a -80 °C freezer for later use with total RNA isolation protocol.

> **Note on removing yeast from filter:** when removing the yeast cells from the nitrocellulose filter, add AE buffer to the filter-containing tube and gently agitate by hand to resuspend the yeast. Pour the AE-yeast into an Oakridge tube to continue with prep. The nitrocellulose is brittle when it is frozen, so be gentle.