

## Yeast Fermenter Runs

Some notes (courtesy of Jessica Dermody)

**NOTE 1:** Conditions were derived from use of a 12L fermenter. Yields assume you'll be using non-selective rich media (1.5 x YPD-S) and a WT-like strain.

### *Fermenter Set-up -*

- 1.1** Make up a 1.5xYP powder mix (per L: 30g Bacto Peptone, 15g Yeast Extract) supplemented with (per L) 0.25g Tryptophan, 0.25g Adenine (for standard media composition see the appropriate protocol at <http://mckeogh.googlepages.com/protocols>)

**NOTE 2:** Mix the YP, Trp, and Ade together in a 2L bottle the evening before. DO NOT add glucose as it is hygroscopic and will form chunks in the mix making it *extremely* hard to pour through the little hole at the top of the fermenter.

- 1.2** Morning of, rinse the (hopefully properly cleaned by the previous user) fermenter thoroughly with dH<sub>2</sub>O. Immediately before adding the media powder from above to the fermenter mix in glucose (30g/L). Add 1L dH<sub>2</sub>O to the 2L bottle and shake to mix. Pour into fermentor and rinse out remaining powder. Make to desired volume with dH<sub>2</sub>O.
- 1.3** Turn on fermentor agitator and autoclave (maintain 121°C) for 20 – 25 min (depending on the fermentor-specific instructions). Don't do more than 30 min or you start caramelizing the glucose which will depress the yeast.

### *Culture Set-up -*

- 2.1** Day before fermenter run: set up a 10mL culture of yeast strain in selective media or YPD, your choice (see **NOTE 1**). Grow overnight with shaking at 30°C.
- 2.2** Morning of fermenter run: subculture 5mL of overnight into 100mL YPD / selective media. Grow for about 4 hour at 30°C. You want an OD<sub>600</sub> of ~ 0.3 when you inoculate.
- 2.3** Inoculate fermenter with entire 100mL culture from **Step 2.2** when the media has reached 30°C (fermenters vary on how long it takes to heat up to autoclave and cool down to 30°C, so time your cultures carefully).
- 2.4** **Standard 12L Run:** Typically I end up inoculating the fermenter between 2pm and 3pm. The next morning, add 300ml of 40% glucose. This gives the yeast an extra kick towards the end of log phase.
- 2.5** **Standard 12L Run:** Collect yeast at an OD ~8.0, which usually occurs between 4pm and 6pm on day 2 of growth (28 hour run). Collect yeast by centrifugation, wash them once in ddH<sub>2</sub>O, transfer into 50ml Falcons and collect by centrifugation. Determine the wet weight of the pellets and store them at -80°C indefinitely.

- 2.7 Standard 12L Run:** Final yields are between 130 – 150g wet weight from a 12L fermenter run using the BY4741 background strain (derived from S288C). **When using W303 strains yields are higher: 200 – 250g wet weight.**