Serial growth experiment

This is a general protocol for measuring growth of yeast after *E.coli* growth in the same media.

All work is performed on a sterile bench.

E. coli growth

Day 1

1. Inoculate 5 ml YNB pH ~7 media with E.coli (either from plate or liquid culture).
2. Incubate over-night with shaking at 37°C.

Day 2

3. Inoculate 25 mL YNB -Trp pH~7 media with overnight culture to a final OD_{600} of ~0.10 (use a 100 mL conical flask or 250 ml for 50 mL culture).
4. Incubate for 3 hours at 30°C with shaking.
5. Add IPTG to a final concentration of 1.0 mM to induce gene expression.
6. Incubate over-night with shaking at 30°C.

Day 3-5

- At desired time intervals:
    - Measure OD_{600}
    - Take a sample and remove *E. coli* from this as described below.

E. coli removal

1. Spin sample for 5 min at max speed, transfer the supernatant to a fresh tube and repeat 2-3 times until no pellet can be seen in bottom of tube.
2. Add 0.02 g glucose per 1 mL sample.
3. Filter the sample through 0.22 µm filter into fresh sterile tube.
4. Store sample at 5°C.

S. cerevisiae growth

Day 1

1. Inoculate 5 ml YNB with a Trp knockout yeast strain (e.g. AM94).
2. Incubate over-night with shaking at 30°C.

Day 2

3. Spin the overnight culture for 5 min at max speed and remove the supernatant.
4. Wash the pellet twice in sterile nuclease free water.
5. Re-suspend the final pellet in 5 mL nuclease free water.

6. Inoculate each YNB -Trp medium sample from above with 100 uL of the re-suspended cells (or in a sufficient volume to reach a desired OD_{600}).
7. Incubate at 30°C
8. At desired time intervals measure OD_{600}.
9. Use microscopy to check for possible contamination at the end of the experiment.