Growing OP50 Cultures for Seeding NGMA Plates

1. First locate your supplies:

- Fresh LB streak plate of OP50 (no more than 2 weeks old) - stored in the fridge.
- 2 L Flask(s) with 500 ml sterile LB.

  NOTE: *This may already be prepared, but if not, prepare by autoclaving LB in flasks.*
- 200 ml LB
- 6 cm unseeded NGMA plates
- Plastic 15 ml culture tubes
- Plastic inoculating loops
- Sterile graduated 5 ml pipets and pipettor
- 1.5 ml sterile tubes
- 1 ml micropipet and tips
- Plastic cuvettes and spectrophotometer (Morimoto Lab)

DAY 1: Prepare OP50 5 ml starter culture.

2. Aliquot 5 ml of LB each into 2 plastic culture tubes. Label one “OP50” and the other “LB only” for a contamination control, since there is no antibiotic selection in the media.
3. Inoculate the OP50 tube with one colony of bacteria from the streak plate.
4. Grow the culture overnight (16-18 hours) at 37°C, 225 rpm in the Morimoto lab shared shaking incubator.

DAY 2: Grow OP50 500 ml culture(s) to OD = 0.5-0.6.

5. Make sure there is no growth in the control LB tube, and there is growth in the OP50 tube.
6. Sterilely add 1 ml of the 5 ml culture to the 500 ml LB in the flask, using a 1 ml micropipet, which yields a starting OD of ~0.005.

   NOTE: *The overnight culture will be very close to OD = 2.5, though to verify if desired, measure the OD by diluting the 5 ml culture 10X in LB in 1 ml volume in a clean cuvette. Use an LB blank for referencing.*
7. Incubate the flask(s) at 37°C, 225 rpm in the Morimoto lab shared shaking incubator.
8. Monitor the OD after the first 2.5 hours and stop growth when the culture is at OD = 0.5-0.6 (usually at about 4 to 4.5 hours).
9. Aliquot ~200 ul of the culture into a 1.5 ml tube for seeding to Test Plates.

10. Immediately store the culture(s) in the 4°C room, with a label indicating this is “Andersen lab OP50 for seeding plates.” But don't use the culture until you have tested it on plates for any contamination or growth issues.
11. Spot 6 cm NGMA test plates (2 per culture) with 100 ul of the culture aliquots and place plates at 37°C overnight.
12. The next day, check OP50 lawns on plates for normal growth and no contamination. Indicate on the culture flasks labels when the culture is ready to use.