# Competition Procedure

Once you have cocultures with D0Mx and D1000Mx, you can set up the competitions. Here is what you should do.

Supplies needed:

Microfuge tubes, anaerobic (3 per competition for time 0 and 3per competition for time final), pre-labelled

CCMA tubes (3 per competition), pre-labelled

Needles and syringes

Glovebox

Centrifuge

Incubator

Spectrophotometer

Procedure:

1. Measure the OD of all of your competitor cultures using the Spectrophotometer for background information that can be used for interpretation later, if needed.
2. We are going to start these competitions with the competitor (D1000Mx) as rare compared to the ancestor. One reason to do this is that cultures with D1000Mx tend to have a higher population size compared to those with D0Mx and therefore, if we mixed them easily we might be starting with a culture that is mostly D1000Mx and it might be harder to see evolution of D1000Mx. Thus, in the microfuge tubes, we will add 100 ul of the competitor D1000Mx and 1400 ul of the ancestor control, D0Mx.
   1. Open the ancestor control tube (D0Mx), and use a pipet to remove 1400 ul (1.4ml)and put it in the appropriately labeled microfuge tube (you could also use a needle and syringe if preferred). Add 0.1ml of the competitor (D1000Mx) to the same microfuge tube.
   2. Close the microfuge tube and mix it.
   3. Remove 0.2 ml from the microfuge tube and add it to your CCMA tubes.
   4. Repeat steps A-c for each replicate and competition of your experiment.
3. Remove the microfuge tubes from the glovebox and centrifuge them at 10,000xg for 10 minutes.
4. Pour off the supernatant. It is good to have a little bit of liquid left in the tube.
5. Put the tubes in a freezer box that is labelled with your names, the initials KLH, and Coevolution competions time 0. Put the samples in the -80oC freezer.
6. Put your culture tubes in the incubator.
7. Monitor the growth of competitions by measuring their OD 2x/per day.
8. When they reach stationary phase, transfer 0.2mL to fresh media, and remove, centrifuge, and freeze 1.5 mls of culture, as described above in steps 3-5. This is T1. Make sure you have an appropriately labeled box for this timepoint.
9. Monitor the growth again. When cultures hit stationary-phase, remove, centrifuge, and freeze 1.5 mls of culture, as described in step 7. This is time final,ideally placed in a separate box. No need to further transfer