# Starting Co-Evolution Project Procedure

Supplies needed:

Fresh CCMA media

Freezer stocks of desired co-cultures

Labels

Ice

Syringes and needles

Incubator

Spectrophotometer

Alcohol

1. Obtain desired freezer stocks of co-cultures from -80° freezer and put on ice.
2. Label each CCMA tube.
3. Warm freezer stocks to room temperature when ready to inoculate and sterilize tops of tubes with alcohol.
4. Using a needle and syringe draw up 0.2mL from the freezer stock, careful to reduce oxygen intake.
   1. Flick the syringe to aggregate oxygen bubbles at the needle end.
   2. Depress the syringe to remove oxygen bubbles
5. Insert needle into appropriate CCMA tube and inoculate.
   1. Gently mix CCMA tube.
6. Place in an incubator.
7. Measure OD daily with a spectrophotometer until it reaches 0.300nm.
8. Complete transfers as many times as desired.