Producing Anaerobic Liquid Media:

Materials Needed:

Anaerobic tubes with rubber stoppers and seals
TSB media
Disposable Needles
Compressed Gas Cylinder containing 5% CO_2 balanced with N_2 (1)

Protocol (estimated time 2 hours):

1. Create a stock of 200mL - 500mL TSB in the anaerobic bottle and autoclave
2. While the media is still hot (all gases are located in the headspace and no oxygen dissolved in the media), insert two needles in a stopper and and flush the flask with 5% CO_2 balanced with N_2 gas for six minutes.
    a. After six minutes, remove the outflow needle so that the gas is still pumping into the flask and quickly remove the inflow needle. Removing the outflow needle first ensures that the flask is slightly pressurized, however the inflow needle must be removed as quickly as possible to avoid high pressure in the flask.
3. Allow the TSB to cool to room temperature for future use.

Growing Anaerobic Bacteria in Test Tubes:

Materials Needed:

Serum bottle filled with flushed TSB
Hungate tubes
Compressed Gas Cylinder containing 5% CO_2 balanced with N_2 (1)
Syringe and disposable Needles
1.5mL Eppendorf Tubes
Pipets
70% ethanol

Protocol (estimated time 2 hours):

1. Flush empty sealed hungate test tubes with 5% CO_2 balanced with N_2 for two minutes.
2. Aseptically insert a 10mL syringe into the flask and withdraw approximately 5mL TSB.
    a. Perform aseptically by spraying the top of the anaerobic flask with 70% ethanol, wiping it with a kimwipe, and burn the top prior to inserting any syringe.
    b. Eject air bubbles that may be present in the syringe.
3. Aseptically inject approximately 5mL of TSB into the test tube. This may be difficult as you will be pressurizing the tube.
4. Get a 1mL syringe and withdraw approximately 1mL TSB from the test tube and inject it into a sterile Eppendorf tube.
5. Dab a colony with a pipet tip and dispense it into the TSB in the Eppendorf tube. Be sure to inject and withdraw a couple times to ensure cells are in the TSB.

6. Take the TSB containing the cells with the 1mL syringe and eject any air bubbles that may have formed.
7. Aseptically inject the TSB with the cells into the flask.
8. Place on a shaker in 37°C to grow.

References

1. Kwong, W., Engel, P., Koch, H., and Moran, N. (2014). Genomics and host specialization of honey bee and bumble bee gut symbionts. *Proceedings of the National Academy of Sciences, 111, 11509-11514.*