**Growing bacteria in Super Broth (“thick food”)**

**Prepare Super Broth:**

Add 1 bottle (100 ml) K-orthophosphates to each bottle of Super Broth (900 ml)

**Prepare starter culture:**

Add 30 ml of Super Broth (plus K-orthophosphates) to a 100 ml flask. Inoculate HB101 from plate in cold room. Use sterile flask and work under sterile conditions.

Incubate for several (8 hours) hours at 37 °C, shaking (200 rpm).

**Prepare over night cultures:**

Add 1 L Super Broth (plus K-orthophosphates) to a 2.5 L sterile flask.

Add ca. 5 ml starter culture (check that bacteria have grown!) to each 1L medium.

Incubate over night at 37 °C, shaking (180-200 rpm). You may need to book an incubator to accommodate several flaks.

**Prepare the thick food:**

Transfer cultures to 1000 ml plastic bottles for centrifugation.

Use the large Sorvall RC 3B Plus centrifuge, rotor H-6000.

Spin cultures at 4000 rpm, 15 minutes, 4 °C. Discard supernatant and repeat centrifugation with the other half (500 ml) of cultures.

Discard supernatant and re-suspend pellet in 25-30 ml of H2O (sterile). Or b-broth. Work under sterile conditions to avoid contamination of bacteria.

Seed culture using electronic pipette on low speed by dropping one droplet at a time, 2 ml in total on each of 14mm plate. The rationale behind this is that worms like the edges of a bacterial lawn and seeding droplets increases the total length of edges.

A 14mm plate can grow up to 80,000 animals.

The bacteria can be frozen for later seeding.

A postdoc in the lab says to freeze resuspended bacterial while another postdoc says to freeze pellet.