



Working

## Growing bacteria in Superbroth ("thick food")

Version 1

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### ABSTRACT

This protocol shows you how to prepare "thick food" to grow *C. elegans* at higher densities.

### MATERIALS

NAME ▾	CATALOG # ▾	VENDOR ▾
Potassium Dihydrogen Orthophosphate Certified AR for Analysis Fisher Chemical	P/4800/53	Fisher Scientific
Superbroth for bacterial culture	<a href="#">View</a>	

### SAFETY WARNINGS

This protocol requires a Bunsen burner. Do not wear gloves around a Bunsen burner as they might catch fire and hurt you.

### BEFORE STARTING

Make sure you have superbroth medium and potassium orthophosphates. If you work at the Gurdon Institute, you can order these solutions from the media kitchen and a week notice is ideal.

#### Prepare Super Broth

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

#### Prepare starter culture

- 2 Add 30 ml of Super Broth (plus K-orthophosphates) to a 100 ml flask. Inoculate *Escherichia coli* HB101 (also works with *E. coli* OP50, *Acinetobacter schindleri*, *Bacillus pumilus* and *Pseudomonas fragi* although not all these bacteria grow at 37 °C) 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). This culture can also be left to grow overnight.

#### Prepare overnight cultures

- 3 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 flasks.

#### Prepare the thick food

-Transfercultures to 1000 ml plastic bottles for centrifugation. Use the large SorvallRC 3B Plus centrifuge, rotor H-6000.

- 4
- 5 -Spincultures at 4000 rpm, 20 minutes, 4 °C.
- 6 Discardsupernatant and re-suspend pellet in 25-30 ml of H<sub>2</sub>O (sterile). Or b-broth.Work under sterile conditions to avoid contamination of bacteria.
- 7 Seedculture 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 thatworms like the edges of a bacterial lawn and seeding droplets increases thetotal length of edges.
- 8 A 14mmplate can grow up to 80,000 animals.
- 9 Thebacteria can be frozen at -20 C for later seeding. A postdoc in the lab says to freeze resuspended bacterial while another postdoc says to freeze pellet. I personally froze the resuspended bacteria so that I can seed them immediately after defrosting.



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