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Culturing C. elegans worms in liquid culture

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We use this protocol and it's working

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Abstract

Growing C. elegans worms in liquid culture

Materials

MATERIALS

- 100000-1000000 arrested L1 worms
- S Basal solution
- 1 M Potassium Citrate
- Trace metals solution
- 1 M Calcium Chloride
- OP50 pellets
- conical bottom centrifuge bottles

1 Copy-pasted protocol from the Wormbook. Got a protocol from Asia Kosalka. liquidcultureAsia.docx Added my notes below.

2 *Reagents*

3 S Basal [5.85 g NaCl, 1 g K_2 HPO_4, 6 g KH_2PO_4, 1 ml cholesterol (5 mg/ml in ethanol), H_2O to 1 litre. Sterilize by autoclaving.]

4 1 M Potassium citrate pH 6.0 [20 g citric acid monohydrate, 293.5 g tri-potassium citrate monohydrate, H_2O to 1 litre. Sterilize by autoclaving.]

5 Trace metals solution [1.86 g disodium EDTA, 0.69 g $FeSO_4$•7 H_2O, 0.2 g MnCl2•4 H_2O, 0.29 g $ZnSO_4$•7 H_2O, 0.025 g $CuSO_4$•5 H_2O, H_2O to 1 litre. Sterilize by autoclaving. Store in the dark.]

6 1 M $CaCl_2$ [55.5 g $CaCl_2$ in 1 litre H_2O. Sterilize by autoclaving.]

7 S Medium [1 litre S Basal, 10 ml 1 M potassium citrate pH 6, 10 ml trace metals solution, 3 ml 1 M $CaCl_2$, 3 ml 1 M $MgSO_4$. Add components using sterile technique; do not autoclave.]

8 4 large plates of *C. elegans*, just cleared of bacteria

9 concentrated *E. coli* OP50

10 *Methods*

11 Add 250 ml S Medium to a sterilized 1-2 litre flask.

12 Inoculate the S Medium with a concentrated *E. coli* OP50 pellet made from 2-3 litres of an overnight culture.

13 Wash each of 4 large plates of *C. elegans* (just cleared of bacteria) with 5 ml S Medium and add to the 250 ml flask.

14 Put the flask on a shaker at 20°C. Use fairly vigorous shaking so that the culture is well oxygenated.

15 Cultures should be monitored bychecking a drop of the culture under the microscope. If the food supplyis depleted (the solutionis no longer visibly cloudy) add moreconcentrated *E. coli* OP50 suspended in S Medium. When there are many adult animals in each drop, the culture is ready to be harvested. This isusually on the 4^{th} or 5^{th} day.

16 Put the flask on ice for 15 minutes to allow the worms to settle.

17 Aspirate most of the liquid from the flask.

18 Transfer the remaining liquid to a 50 ml sterile conical centrifuge tube and spin for at least 2 min at 1150 × g to pelletthe worms. Young larvae may take longer than 2 min to pellet.

Conical bottom plastic centrifuge bottle

19 Aspirate the remaining liquid.
The worms harvested from growth in liquid culture areusually longer and thinner than those grown on petri plates, and tendto hold their eggs. The number of adults, larvae and eggsobtained depends on the strain of worms used, the amount of bacteriaprovided and the length of time the culture is grown.Freshly cleared cultures will yield worms equal to half the weight ofthe bacteria used (**Lewis and Fleming, 1995**).

Notes

20 Add arrested L1 worms to the liquid culture. This has several benefits:
- minimises contamination
- synchronised growth which means that the bacterial depletion will occur at a more predictable rate