

Preparation of synchronized cultures

Synchronously growing animals can be prepared by first axenizing the nematode stock, then transferring the eggs to a new plate and collecting animals at different larval stages.

Axenization of the nematode stock:

1. Young gravid adult worms from a 3-day old plate at 25°C are floated off in sterile M9 buffer or water and transferred to a sterile disposable 15 ml falcon tube. (To avoid contamination, no effort should be made to wash off eggs stuck to the plate).
2. Worms are spun down 2 mins at 1000 rpm in a tabletop clinical centrifuge.
3. The supernatant is removed and the pellet is rinsed by further resuspending in fresh buffer or water.
4. The animals are once again spun down and the supernatant is removed.
5. 7-10 mls of sodium hypochlorite solution (2ml of fresh chlorox bleach i.e. 4-6% sodium hypochlorite and 5 mls of 1 N NaOH to axenize one plate) is added to the tube and allowed to stand for 10- 15 mins at room temp with brief and careful vortexing every few minutes to resuspend the worms. Within a few minutes adult worms will dissolve leaving a hazy solution of eggs liberated from their carcasses.
6. The eggs are then collected by centrifugation at 1000 rpm for 2 mins.
7. The egg pellet is then washed twice by resuspending in M9 buffer and collected by centrifugation.
8. The washed eggs are resuspended in 2 mls of M9 buffer and shaken for 17-22 hrs at 25°C
9. The eggs hatch and L1 larvae are arrested at this stage due to starvation.
10. To avoid the effects of dauer pheromone accumulated during starvation starved L1 should be washed once with M9 buffer and pelleted by centrifugation at 1000 rpm for 2 mins .
11. The final pellet should be resuspended in a small volume (less than 0.5 ml) for transfer by pasteur pipet to an NGM plate seeded with OP50.
12. The transfer plate will have many newly hatched worms including some worms hatched and disfigured at the conclusion of the hypochlorite treatment.
13. By harvesting at appropriate time intervals, synchronous larval stages and adults can be collected.