UNION BIOMETRICA
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Sample Preparation Protocol SP-03
Sucrose Float Technique for *C. elegans* (nematode)

Scope

The intended use of this protocol is for rapid cleaning of a culture of *C.elegans*. Bacteria, dead worms, and other dense objects or contamination will be separated from the viable worm population. This protocol can also be used in all procedures requiring rapid loading of culture arrays with precise numbers of animals. No size selectivity is accomplished using this technique. This technique should be used prior to sampling them on the COPAS instruments.

Materials

M9 buffer with 0.01% Triton X-100 (See Sample Preparation Protocol SP-06)
180µm nylon mesh (Millipore Corp. catalog number NY8H04700)
Water
60% Sucrose solution
15 ml conical tube
Pipette 10 ml or Pasteur Pipette
Monodisperse solution of *E.Coli*

Procedure

Wash *C. elegans* off agar medium using M9 buffer with 0.01% Triton X-100. In the event of heavy contamination of the culture, or if liquid medium is used, the sample should be passed through a 180 µm nylon mesh to remove larger clumps of eggs and debris.

Pellet worms in a 15 ml conical tube by centrifugation (at low speed) or by settling. Re-suspend the pellet of worms in 3 ml of water. The water will make the worm preparation more buoyant.

Make a stock solution of 60% sucrose for use with this and other methods. KEEP THIS STOCK REFRIGERATED.

Using a 15 ml conical centrifuge tube, mix equal volumes of the washed worm prep and the stock 60% sucrose solution to yield an approximate sucrose concentration of 30%, and a final fluid volume of 7 to 10 ml. Keep the sample cold to inhibit thrashing of the worms.

Spin in a cold centrifuge for 5 minutes at a high speed.

Aspirate floating worms using a Pasteur or transfer pipette and expel into a clean conical centrifuge tube. Wash harvested worms with distilled water and centrifuge. Repeat once more. This will remove excess sucrose from the worms. Suspend the harvested animals in 10 ml of M9 buffer, or other diluent.

Determine the concentration of animals/ml using a microscope. Adjust the concentration of worms to between 500 and 2000 per ml, by adding diluent.

NOTE: For rapid loading of culture arrays a semi-staged sample with a concentration of 1000 to 2000 worms / ml is recommended.

NOTE: For precise selection of animals of a specific size from a mixed population, a semi-staged sample with a concentration of 500 to 1000 worms / ml is recommended.