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Sample Preparation Protocol SP-04
Sucrose Sedimentation Technique for *C. elegans*

Scope

The intended use of this protocol is for cleaning and semi-staging of a mixed culture of *C. elegans* (nematode). This technique should be used to prepare worms for all procedures requiring high precision of specific size organisms and all procedures requiring rapid loading of culture arrays with precise numbers of worms prior to processing 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
Centrifuge
Microscope
Pipette 10 ml or Pasteur Pipette
10 µl eppendorf pipette
Monodisperse solution of *E.coli*

Procedure

Wash animals 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 by centrifugation (at low speed) or by settling, and re-suspend in 3 ml of M9 buffer, or other diluent.

Make a stock solution of 70% sucrose for use with this and other methods. KEEP THIS STOCK REFRIGERATED.

From the stock 70% sucrose, make 10 ml each of 7% and 3.5% sucrose solutions using distilled water. Keep working solutions cold (approximately15ºC) to avoid thrashing of animals.

Set up a step gradient in a 15 ml conical tube. First add 3 ml of 7% sucrose to the bottom of the tube. Next, layer 6 ml 3.5% sucrose on top of the 7% sucrose, avoiding mixing of the two solutions. Finally, layer the 3 ml sample on the 3.5% sucrose, again avoiding mixing.

Allow worms to settle at room temperature for approximately 15 minutes. Large worms will enter the 7% sucrose layer and form a pellet. Smaller animals will settle into the middle layer at a rate determined by both size and density. Removing either faster or slower settling worms into a clean tube can produce some size selectivity of this middle layer.

Decant the layers separately, and use as needed. The top layer should be discarded since contains mainly dead worms and empty cuticles. Wash harvested animals with distilled water and centrifuge. Repeat once more. This will remove excess sucrose from the animals.

Suspend the washed *C. elegans* in 10 ml of M9 buffer, or another diluent. Adjust a 10 μl eppendorf pipette to aspirate 5 μl. Mix the worm suspension, and aspirate 5 μl of sample. Expel the 5 μl of sample onto a microscope slide. Repeat aspiration of 5 μl onto a microscope slide, twice. (TOTAL: 3 DROPS ON SLIDE EACH CONTAINING 5 μl OF WORM SUSPENSION.)

Using 4X or alternate objective, count the number of worms and eggs present in the 15 μl (N). Calculate the concentration of animals/μl using the following formula: ANIMALS/μl = N / 15

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 animals/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 animals/mL is recommended.

NOTE: If a large sample batch is to be run over a long period of time, a low concentration of *E. coli* can be added to the sample. If this is required, the *E. coli* must be monodisperse.

Questions?

For further information, please contact Union Biometrica, Inc. directly at 617.591.1211 or email your questions to appsupport@unionbio.com