NemaMetrix

C. elegans Synchronizer System

Protocol for synchronization of L1 nematodes

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Warning:

- Make sure to protect the filters when not in use by placing a 90mm petri dish cap on each side.
- When working with pipettes, be careful not to puncture the filters.
- Before use, please read the general instructions.

Preparation:

- Sterilize the filters, funnel, crystalizing dish, etc. with 70% ethanol and rinse once with sterile M9/S-media.
- For optimal yield and synchronization, the majority of the *C. elegans* culture should consist of gravid adults and eggs.

Cleaning Before First Use:

1. Soak filters in 0.5-1M NaOH for 30 minutes up to 1 hour.
2. Move the filters to soak in distilled water for 30 minutes.
   - *Caution: Ensure that the water is free of traces of dissolved metals . Metals can react with the nickel alloy in the filters and cause scaling.
3. Rinse the filters twice using a wash bottle of distilled water.
4. Cover the filters with a petri dish lid to avoid damaging the mesh.
5. Let the filters dry in an oven for 1 to 2 hours.

Stabilization of Gravid Population

1. Place funnel in holder.
2. Attach about 2 inches of the tubing to the funnel spout and clamp with Hemostat locking clamp.
3. Pla ce the end of the tubing in a waste container or glass dish.
4. Place the “Stabilization Filter" in the funnel.
5. Select a population of worms (from NGM plate or liquid culture) with a high number of gravid adults. Ensure the population is well fed for optimal L1 yield.
6. Transfer worms to "Stabilization Filter" with glass pipet.
   - *Caution: Take care not to touch filter surface with pipet. Glass pipets can damage the filters!
7. Close the waste tube using the Hemostat clamp.
8. Rinse the worms with at least 300 mL of M9 or S Basal.
9. Let the worms sediment for 5-10 minutes
10. Slightly tilt the filter, washing the adults and eggs to the lower corner. Pipet them out and transfer them to the "Harvest Filter" *(see next step "Harvesting L1's in liquid")*
11. Release Hemostat clamp to drain waste buffer and larval worms.

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Harvesting L1s in Liquid

1. Place 10mL M9 or S Basal into a clean glass dish.
2. Place the “Harvest Filter" in the dish with clean buffer.
3. Transfer the contents of the Stabilization Filter" into the "Harvest Filter" with a glass pipet.
4. Cover the top of the filter with a petri dish to protect from dust.
5. Leave the filter in the dish for 15 minutes up to 12 hours (depending on level of synchronization required). L1s will hatch and pass through filter.
6. Slowly remove "Harvest Filter" and set aside.
7. Transfer contents of dish to 2 or more conical tubes.
8. Use a centrifuge to spin down L1s (5 minutes at 1200-2000g).
9. Transfer worm pellet to seeded NGM plate or Liquid culture.

Cleaning After Use:

1. Rinse from the underside of the filter mesh with a wash bottle and ethanol.
2. Rinse the wall of the filter with ethanol.
3. Rinse the filter mesh and walls with distilled water.
4. Repeat Steps 1-3 2 more times.
5. Cover the filter mesh with a petri dish to avoid damage.
6. Set filters in a drying oven for 1-2 hours.

To remove scaling and debris trapped in filter:

1. Soak filters in 0.5-1M NaOH for 3 hours to overnight.
2. Move the filters to soak in distilled water for 30 minutes.
3. Gently swirl the filter in a small(1L) 40kHz ultrasonicator for 60-120 seconds to eliminate scaling and build up.
4. Rinse the filters using a wash bottle of distilled water.
5. Examine filters under microscope. If build up remains, repeat Steps 8 and 9 until clear.
6. Cover the filters with a petri dish lid to avoid damaging the mesh.
7. Set filters in a drying oven for 1-2 hours.