Adult

Separation

Protocol

Using CES-700/5000

Warning:

- Make sure to protect the filters when not in use.
- When working with pipettes, be careful not to puncture the filters.
- Before use, please read the general instructions.

Preparation:

- Sterilize the filters, funnel, crystallizing dish, etc, with 70% ethanol and rinse once with sterile, demi water or S-Medium.

This protocol is written to separate Adults from offspring *

CES-700 Protocol:

A: Separating Adults from offspring

1. Place the "Stabilization filter" in a large (200mL or more) crystalizing dish.
2. Transfer the worms to the "Stabilization Filter" with glass pipet.
3. Rinse the worms in the stabilization filter with sterile water or S Medium in order to remove most debris, etc.
4. Add ~10ml S Medium into a clean ~20mL glass crystalizing dish. A 50mm crystalizing dish as supplied can be used.
5. Slightly tilt the dish and filter and slowly place the "Stabilization filter" and its content in the dish with M9/ S-media. Make sure there is no air trapped under the filter! There should be a few millimeters of S Medium above the filter surface.
6. Let the worms sediment for 5 up to 15 minutes (or even longer).
7. Tilt the "Stabilization Filter" and carefully wash with for example a pipet and clean media the adults to one side of the filter surface.
8. Pipet the adults from the filter and optional transfer to conical tube(s).
9. Optional: use centrifuge to spin down L1's (~5 minutes at 1200-2000g)
10. Transfer worm (pellet) to seeded NGM plate or liquid culture.

CES-5000 Protocol:

A: Separating Adults from offspring

1. Place funnel in holder.
2. Attach tubing to funnel and place end of tubing in a (waste) container / receiver.
3. Place the “Stabilization filter” in the funnel.
4. Transfer the worms to “Stabilization Filter” with glass pipet.
5. Rinse the worms in the stabilization filter with sterile water or S Medium till most debris is removed.
6. Close/pinch the silicone waste tube using a Hemostat clamp or similar device.
7. Fill the stabilization filter and funnel with S Medium a few (~5mm) millimeters above the filter surface.
8. Let the worms sediment for 5 up to 15 minutes.
9. Tilt the “Stabilization Filter” and carefully wash with a pipet and clean media the adults to one side of the filter surface.
10. Pipet the adults from the filter and optional transfer to conical tube(s).
11. Optional: use centrifuge to spin down L1’s (~5 minutes at 1200-2000g)
12. Transfer worm (pellet) to seeded NGM plate or liquid culture.

Remark: (!!)

it is recommended to have a 'tight' synchronized population of Adults , i.e. make sure when using the CES protocol for 'Adult separation' that there are no L3, L4 and young adults as they may not pass through the first (Stabilizing) filter as the aperture size of this filter is approximately 20um. L3 may still pass through, however this is not guaranteed as the average width of an L3 (from NGM plates) is estimated at 22.2 ± 0.3 µm. This is also why it is recommended to repeat the 'Adult separation' cycle every 24 hours. Any longer and this may cause off-springs to grow too large ">L2" to effectively be separated. You should test this maximum time window as this will also depend on temperature and food availability.

Cleaning / descaling the filters:

1a Soak filters in a 0.5-1 M NaOH for 30 minutes up to an 1 hour.

1b Alternative: use a small 2 liter 40 Hz ultrasonic cleaner, filled with 0.5-1 M NaOH, and clean the filters for approximately 5 minutes. The ultrasonic cleaner will also help descale filters if this becomes necessary.

2 Soak / rinse filters in distilled / demi water.
Caution: do not use standard tap water as this typically contains dissolved salts and metals, as this will react to the nickel alloy and cause scaling.

3 Sterilize the filters with 70% isopropyl alcohol, either use a spray or submerge the filters completely.

4 Let the filters dry in a laminar flow hood.

5 Cover the filters with a petri dish lid.

If there is 'scaling' NEVER use an acid solution as it will have the potential of damaging the filters. Instead, use a small 40khz ultrasonic cleaning device with a 'general purpose' cleaning solution that has pH of 7 or higher. If not sure just use demi-water with a drop of general-purpose detergent and gently move / tilting the filter for 1 to 2 minutes in the ultrasonic cleaning device. To verify the result check the filter before and after under a microscope

Guidelines when working with the CES:

In order to avoid contamination, always try to work in a laminar flow hood if possible

Contamination: (Cleaning Buffer)

The source (plates or liquid) can be a suspect of contamination. If this is the case, then the following Cleaning Buffers could/should be used.

Cleaning buffer 50 mL: M9 + Streptomycin + Nystatin –solution :

1 Streptomycin stock 100 mg/mL
Add 1 gram of Strep to 10 mL of dH2O, filter sterilize the solution and store at 4˚C

2 Nystatin suspension 10 mg/mL
For 50 mL, add 0.5 gram Nystatin in 70% ethanol in dH2O (Shake before use) store at 4˚C

3 Prepare 50 mL of sterile M9 buffer
add 50 µL Streptomycin stock 100 mg/mL and 50 µL Nystatin suspension 10 mg/mL

4 Transfer the L1's to a 50 mL tube and add 10 mL cleaning buffer, incubate for 5 minutes.

Protocol revisions, updates and remarks:

We are continuously working on the improvements of the protocol with input and suggestions from users of the system. As such, we encourage users to share with us any suggestion / improvements, as we want to share this with other users in the *C. elegans* community.

Latest version:

The latest protocols, documentation, demo video can be downloaded at https://www.nemasync.com/documentation

Ver. CES_Adult_Seperation_v1c