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Preparation of staged *Caenorhabditis briggsae* and *Caenorhabditis tropicalis* embryos using size filtration

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Abstract

Bleach synchronization is the traditional method to obtain staged embryos from *Caenorhabditis* nematodes. A previously published size filtration protocol optimized for *Caenorhabditis elegans* reduced bleach exposure and yielded more synchronized embryos than bleach synchronization. Here, we show that this protocol can be adapted to collect embryos from other *Caenorhabditis* species. For *Caenorhabditis briggsae*, we used non-ionic detergent to obtain high yields of embryos, which, unlike *C. elegans*, stick to the filter mesh. No modification was required to collect embryos for *Caenorhabditis tropicalis*. Our results demonstrate that the size filtration protocol can be adapted for embryo collection in additional *Caenorhabditis* species.

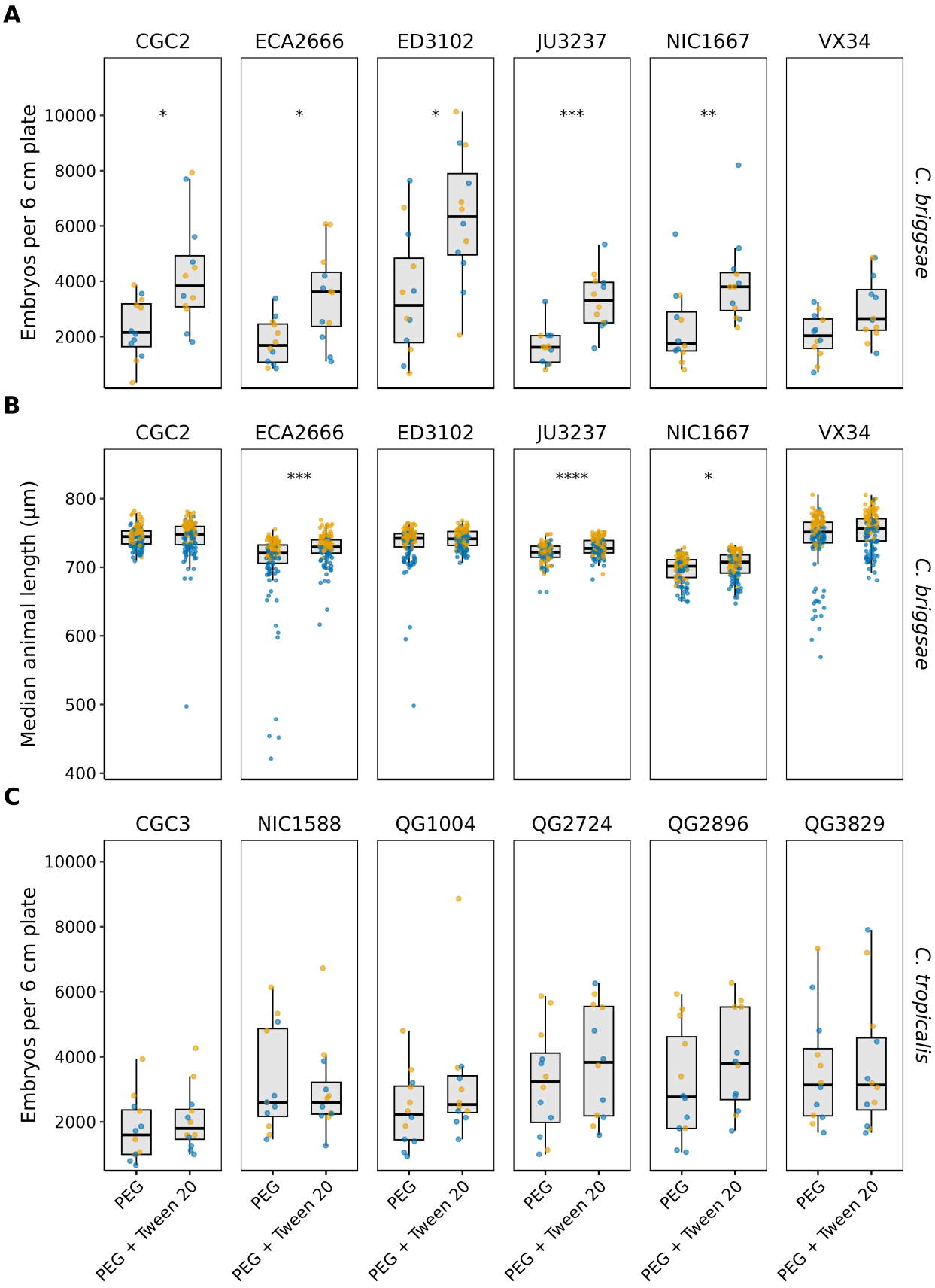


Figure 1. Effects of Tween 20 on embryo yield per 6 cm plate (*C. briggsae* and *C. tropicalis*) and larval development (*C. briggsae*):

(A, C) Box plots show embryonic yield per 6 cm plate obtained using a wash buffer with or without 0.05% Tween 20 for **(A)** *C. briggsae* and **(C)** *C. tropicalis*. The median is represented by the solid horizontal line, with the 25th and 75th quartiles on the bottom and top of the box, respectively. Whiskers extend from the box to the maximum and minimum values that fall within 1.5 times the interquartile range below and above the 25th and 75th quartiles, respectively. Two experimentalists (represented by two colored points) independently collected six biological replicates. **(B)** The box plots show the length of *C. briggsae* L4 larval animals that developed from embryos collected with or without 0.05% Tween 20 in the wash buffer. Each point represents the summarized median animal length of an individual well containing between five and 75 animals. Two experimentalists (represented by two colored points) independently collected a minimum of four biological replicates. The results of the Wilcoxon rank-sum test are shown in the facet for each strain. Asterisks indicate which results are statistically significant (* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$).

Description

Experiments using *Caenorhabditis elegans*, *Caenorhabditis briggsae*, and *Caenorhabditis tropicalis* require populations synchronized at the same developmental stage. High-throughput assays such as genome-wide association studies, drug screens, proteomics, and behavioral assays require many animals at a specific developmental stage. One way to synchronize nematodes is to isolate embryos and grow them to their desired developmental stage. The most common way to obtain embryos is to dissolve the hypodermis of gravid adults using a hypochlorite (bleach) solution (Porta-de-la-Riva et al., 2012). Parameters such as bleaching time and temperature can affect embryo survival (Porta-de-la-Riva et al., 2012). Additionally, exposure to bleach can modify the different eggshell layers (Krenger et al., 2020). To reduce exposure to the harsh conditions, we previously published a protocol that uses mesh filters to isolate synchronized embryos from *C. elegans* (Jhaveri et al., 2025; Jhaveri et al., 2024). Our method exposed *C. elegans* animals to a lower bleach concentration for a shorter period of time and yielded more embryos than traditional bleach synchronization. To expand the application of our size filtration protocol to other nematodes, we applied the protocol to two other selfing *Caenorhabditis* species, *C. briggsae* and *C. tropicalis*. We hypothesized that the embryo dimensions of *C. briggsae* and *C. tropicalis* (Baia et al., 2026; Riddle et al., 1997) would be similar to that of *C. elegans*, and the size filtration protocol could be used on both species.

First, we selected six genetically diverse *C. briggsae* strains and used the previously published size filtration protocol (Jhaveri et al., 2025; Jhaveri et al., 2024). However, embryo recovery was lower with size filtration than with traditional bleach synchronization (CGC2: 838 vs. 1,554; ECA2666: 806 vs. 1,508; ED3102: 628 vs. 2,277; JU3237: 656 vs. 938; NIC1667: 1,4890 vs. 2,649; VX34: 204 vs. 1,147 embryos per 6 cm plate for size filtration and bleach synchronization, respectively). *C. briggsae* embryos adhered to the 20 μm mesh filters and formed a mat that reduced embryo recovery. We hypothesized that the addition of a surfactant could lower the surface tension and increase *C. briggsae* embryo recovery from the mesh filters. To increase embryo recovery, we supplemented the wash buffer (3% polyethylene glycol (PEG) in M9 buffer) with 0.05% Tween 20. We recovered more embryos with the modified wash buffer (3% PEG with 0.05% Tween 20) than using 3% PEG alone (Figure 1A). Embryo yield increased by 48 to 100% for the six *C. briggsae* strains with the modified wash buffer. To determine if Tween 20 affected nematode development, we performed a high-throughput larval developmental assay (Crombie at al., 2026; Shaver & Andersen, 2026; Shaver et al., 2023). We quantified development using animal length because nematodes increase in length as they progress through development. If Tween 20 is toxic, exposure could slow development and result in animals with shorter lengths. Animal length at the L4 larval stage did not differ significantly between embryos collected using a wash buffer with or without 0.05% Tween 20, except for ECA2666, JU3237, and NIC1667 strains where the length is actually longer indicating that Tween 20 has no effect on growth (Figure 1B). These results demonstrated that the use of 0.05% Tween 20 increased the embryo yield without affecting larval development.

Next, we applied the size filtration protocol to *C. tropicalis*, where bleach synchronization has proven challenging and yielded low embryo populations. We collected embryos using bleach synchronization and the previously published size filtration protocol (Jhaveri et al., 2025) and recovered a higher yield with size filtration (CGC3: 1,761 vs 3,849; NIC1588: 3,250 vs 506; QG1004: 2,372 vs 1,262; QG2724: 3,233 vs 773; QG2896: 3,161 vs 1,236; QG3829: 3,566 vs 578 embryos per 6 cm plate by size filtration and bleach synchronization, respectively). We then tested whether embryo recovery increased with the addition of Tween 20, as observed for *C. briggsae*. By contrast, the two wash methods yielded similar embryo counts in *C. tropicalis* (Figure 1C). Because the addition of Tween 20 did not increase embryo yield, we did not test the larval developmental growth in the presence of Tween 20 for *C. tropicalis*. These results suggest that the size filtration protocol can be used for *C. tropicalis* without modification of the wash buffer, as described for *C. elegans*.

We show that the size filtration protocol can be used and modified to collect high embryo yields from two additional *Caenorhabditis* species. The size filtration protocol provides an alternative to bleach synchronization to collect nematode embryos at higher yields and reduce exposure to bleach. By optimizing the size filtration protocol for each species, we could overcome differences in species-specific embryo adhesion properties (Fukushige & Futai, 1985) to ensure high embryo yields for downstream assays . *C. briggsae* embryos could have surface properties that increase their adhesion to

mesh filters compared with embryos of *C. elegans* and *C. tropicalis*. These results demonstrate that the filtration protocol can be optimized across *Caenorhabditis* species by a modification to the wash buffers.

Methods

Nematode maintenance

All strains came from the *Caenorhabditis* Natural Diversity Resource (CaeNDR) (Crombie et al., 2024). *C. briggsae* strains used in the study were CGC2 (O'Connor et al., 2026), ECA2666, ED3102, JU3237, NIC1667, and VX34. *C. tropicalis* strains used in the study were CGC3 (NIC58), NIC1588, QG1004, QG2724, QG2896, and QG3829. *C. briggsae* and *C. tropicalis* strains were maintained at 20°C and 25°C, respectively, for three generations before embryo collection by filtration or bleach. Strains were grown on 6 cm plates of modified nematode growth medium (NGMA) (Andersen et al., 2024). The *Escherichia coli* strain OP50 was used as the food source.

Size filtration to obtain embryos

We collected *C. briggsae* and *C. tropicalis* embryos using the previously published filtration protocol (Jhaveri et al., 2024), with modification to the 3% PEG wash buffer (3% PEG wash buffer with 0.05% Tween 20). Five L4 larval hermaphrodites were transferred to 6 cm OP50-seeded NGMA plates and incubated at 21.5°C for *C. briggsae* or at 25°C for *C. tropicalis* for four days. On the fourth day, the adults were washed from the plates with 2 mL of M9 buffer (3 g of KH_2PO_4, 6 g of Na_2HPO_4, and 5 g of NaCl in 1 L Milli-Q water). The wash step was repeated once. The embryos retained on the OP50 bacterial lawn were collected by adding 2 mL M9 buffer and gently scraping off the embryos using a rubber policeman. The M9 buffer containing the embryos was collected in a 15 mL conical tube and passed through the filtration apparatus (Jhaveri et al., 2024). The connector ring was opened and connected to a vacuum to let the embryo solution pass through the filters. Once all the solution had passed, the filtration apparatus was disassembled. The 20 μm mesh filter, containing most of the embryos and a few L1 larval animals, was inverted and placed on top of a clean 50 mL conical tube. A 4 mL solution of 25% bleach solution (1 mL of bleach solution (2 mL of NaOCl and 0.2 g of NaOH in 10 mL Milli-Q water) + 3 mL of M9 buffer) was added to the reversed side of the 20 μm mesh filter and incubated for 30 seconds to kill the few larvae that were retained on the 20 μm mesh filter. After 30 seconds, 6 mL of 3% PEG in M9 buffer (3 g of PEG in 100 mL of M9 buffer) or 6 mL of 3% PEG with 0.05% Tween 20 (100 mL of 3% PEG + 0.05 mL of Tween 20) was added to the filter. After the solution passed through the filter, the contents of the 50 mL conical tube were transferred to a clean 15 mL conical tube and centrifuged at 3197 g for one minute. The supernatant was discarded, and approximately 10 mL of 3% PEG in M9 buffer or 3% PEG with 0.05% Tween 20 in M9 buffer was added. The 15 mL conical tube was centrifuged at 3197 g for one minute, and the supernatant was discarded. The wash step was repeated once. After the final wash, the embryos were resuspended in 3 mL of K medium (51mM NaCl, 32mM KCl, 3 mM $CaCl_2$, and 3mM $MgSO_4$ in 500 mL Milli-Q water. Filter sterilize using a 0.2 µm filtration unit and add 1.25 µg/mL of unfiltered cholesterol). Embryo titer was determined by counting embryos in five 3 µL aliquots. The assay was carried out in six biological replicates by two experimentalists.

Bleach synchronization to obtain embryos

Five L4 larval *C. tropicalis* and *C. briggsae* hermaphrodites were transferred to 6 cm OP50-seeded NGMA plates and incubated at 25°C for four days. On day four, the adults were collected in a 15 mL conical tube by washing with an M9 buffer. The supernatant was discarded, and 6 mL bleach solution was added. The 15 mL conical tubes were vigorously shaken. As soon as broken bodies were observed under the microscope, the tubes were centrifuged at 254 g for 30 seconds. The bleach solution was discarded and 10 mL of M9 buffer was added to wash the embryos. A total of three M9 washes were performed. After the final K medium wash, the embryos were resuspended in 3 mL of K medium. The embryo titer was determined by counting embryos in five 3 µL aliquots. The assay was performed in three biological replicates.

High-throughput larval developmental assay (HTLDA) in *C. briggsae*

The HTLDA was performed as previously described (Shaver et al., 2023). The *C. briggsae* embryos collected by filtration were diluted to a final concentration of 0.6 embryos per µL in K medium. A total of 50 µL of the embryo solution (approximately 30 embryos) was added to each well of a 96-well plate. To obtain synchronized L1-staged animals, embryos were incubated in K medium overnight at 20°C with shaking at 170 rpm. The next day, the *E. coli* strain HB101 was prepared at an OD_{600} 30 from a OD_{600} 100 stock using K medium. HB101 at a concentration of OD_{600} 100 was prepared as previously described (Widmayer et al., 2022). Kanamycin was added at a concentration of 150 µM to prevent bacterial growth and contamination. Animals grew in the presence of HB101 for 48 hours at 20°C with shaking at 170 rpm. After 48 hours, 50 mM sodium azide was added to each well to straighten and paralyze the animals. Each 96-well

plate was imaged after a 10-minute incubation in sodium azide using a 2X objective in a Molecular Devices ImageXpress Nano microscope (Molecular Devices, San Jose, CA) (Shaver et al., 2023). For developmental analysis, 16 wells (technical replicates) were prepared for embryos obtained by each wash buffer (3% PEG in M9 buffer or 3% PEG with 0.05% Tween 20 in M9 buffer). The assay was carried out in at-least four biological replicates by each experimentalist.

Data processing and analysis

Raw images were processed using the NemaSize v1 pipeline (Li et al., 2026). In brief, the pipeline uses two YOLO26 models to identify and segment animals (Sapkota et al., 2025). The first model is a low-magnification model (YOLO26-WF) used to identify the animals. The second model is a high-magnification model (YOLO26-WS) used to accurately segment the animals and measure the body lengths. The output generated by NemaSize was cleaned and further processed using the custom *R* package *easyXpress* v3 (Nyaanga et al., 2021; Shaver et al., 2023). RStudio (v4.2.1) was used to perform all statistical analyses and figure generation. For development analysis, median animal lengths were calculated for each well.

Scripts and raw data are available as Extended Data.

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Extended Data

Description: Data and code associated with the microPublication, "Preparation of staged Caenorhabditis briggsae and Caenorhabditis tropicalis embryos using size filtration." The repository contains the scripts and associated data used in the study, along with a README file and a CC BY 4.0 license. Version 1.0.0 (November 6, 2026).. Resource Type: Software. File: Micropublication_2026-1.0.0.zip. DOI: 10.22002/dt797-nsy79

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