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Title: Simple and Accessible Egg-Hatching Assays in *Caenorhabditis elegans*: Adaptable Formats for Drug Screening

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Author Questionnaire

1. We have marked your project as author-provided footage, meaning you film the video yourself and provide JoVE with the footage to edit. JoVE will not send the videographer. Please confirm that this is correct.

✓ Correct

2. Microscopy: Does your protocol require the use of a dissecting or stereomicroscope for performing a complex dissection, microinjection technique, or something similar? **YES**

2.1.2, 2.6.2, 2.11.2, 4.1.1, 4.1.3, 4.2.2, 4.2.3, 4.3.2

3. Software: Does the part of your protocol being filmed include step-by-step descriptions of software usage? **NO**

4. Proposed filming date: To help JoVE process and publish your video in a timely manner, please indicate the proposed date that your group will film here: **06/29/2026**

When you are ready to submit your video files, please contact our Content Manager, [Utkarsh Khare](#).

Current Protocol Length

Number of Steps: 24

Number of Shots: 55

Interviews

INTRODUCTION:

~~REQUIRED: Summarize your research focus and the main questions it aims to answer.~~

- 1.1. **Guillermina Hernando:** We develop simple and scalable *C. elegans* egg-hatching assays to evaluate compound effects on embryonic development.
 - 1.1.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera.

~~Describe the research gap or limitation in existing methods and how this protocol addresses them.~~

- 1.2. **Alcibeth Pulido Carrasquero:** Existing methods lack standardized, adaptable workflows; this protocol provides an accessible, standardized approach suitable for both low- and high-throughput screening.
 - 1.2.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera.

CONCLUSION:

~~What does this protocol allow researchers to study or measure?~~

- 1.3. **Guillermina Hernando:** This protocol measures egg hatching and embryonic responses to drugs using complementary experimental formats.
 - 1.3.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera.

~~Describe the most important consideration or challenge when performing this protocol.~~

- 1.4. **Alcibeth Pulido Carrasquero:** The key consideration while performing this protocol is precise egg preparation and uniform exposure conditions.

- 1.4.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera.

~~How can future studies build on this work? Describe the questions or research directions that can be investigated next.~~

- 1.5. **Guillermina Hernando:** Future studies can combine these assays with imaging, automation, and mechanistic analyses to accelerate drug discovery.

Protocol

2. Obtaining Synchronized *Caenorhabditis elegans* Eggs

Demonstrator: Alcibeth Pulido Carrasquero

- 2.1. Start with 3 to 5 nematode growth medium plates containing confluent cultures of gravid adults [1]. Select plates containing large numbers of gravid worms and visible eggs indicating active reproduction [2].
 - 2.1.1. WIDE: Talent arranging 3 to 5 nematode growth medium plates containing confluent cultures of gravid adults on the bench. NOTE: 2.1.1., 2.2.1 and 2.2.2 are filmed in a single shot)
 - 2.1.2. SCOPE: 2.1.2-(2)-agar-plate.mp4: 00:14-00:20
- 2.2. Add distilled water to each plate [1] and gently wash the worms into a 15-milliliter conical tube. Avoid scraping the agar surface to minimize debris [2].
 - 2.2.1. Talent adding distilled water to each plate.
 - 2.2.2. Talent gently washing worms from the plates into a 15 milliliter conical tube.
- 2.3. Centrifuge at 266 *g* for 3 minutes to pellet the worms [1]. Remove the supernatant containing bacteria, then repeat washing the worm 3 times [2].
 - 2.3.1. Talent placing the 15 milliliter conical tube in the centrifuge.
 - 2.3.2. Talent removing the supernatant from the tube.
- ~~2.4. Repeat the washing three times while resuspending the pellet gently after each wash to improve the removal of residual bacteria [1].~~
 - ~~2.4.1. Talent gently resuspending the pellet in the tube. NOTE: Not filmed~~
- 2.5. To isolate the eggs, resuspend the worm pellet in a freshly made bleaching solution right before use to maintain effectiveness [1]. Vigorously agitate the tube to promote dissolution of the adult bodies and release of the eggs [2]. Mix continuously during incubation to ensure uniform exposure, and do not exceed 5 minutes of incubation [3].
 - 2.5.1. Talent resuspending the worm pellet in the freshly prepared bleaching solution.
 - 2.5.2. Talent vigorously agitating the tube and places it in the tube holder.
 - 2.5.3. Talent continuously mixes the tube during incubation.
- 2.6. ~~Monitor the reaction closely and do not exceed 5 minutes of incubation [1].~~ Visually confirm that the adult bodies are mostly dissolved, while the eggs remain intact [2]. NOTE: The VO for the deleted shot is added concisely in the previous shot
 - ~~2.6.1. Talent observing the reaction tube. NOTE: Not filmed~~
 - 2.6.2. SCOPE: 2.6.2.mp4: 00:05-00:10

- 2.7. Monitor the reaction and stop it after 5 minutes by adding cold sterile distilled water to a final volume of 14 milliliters to prevent egg damage [1]. Cap the tube and invert it gently several times to quickly dilute the bleaching solution, preserving egg viability [2].
- 2.7.1. Talent adding distilled water to the tube.
- 2.7.2. Talent placing the cap on the tube and gently inverting the tube several times.
- 2.8. Then, centrifuge at 266 *g* for 3 minutes [1] and carefully discard the supernatant [2].
- 2.8.1. Talent placing the tube in the centrifuge.
- 2.8.2. Talent carefully discarding the supernatant from the tube.
- 2.9. Wash the egg pellet three times with sterile distilled water to completely remove residual bleach, while ensuring thorough resuspension between washes [1]. Then, resuspend the eggs in 5 milliliters of 60% sucrose with 5 milliliters of distilled water [2]. Collect the eggs from the sucrose gradient ring and wash them with sterile water [3]. Centrifuge the tube at 266 *g* for 5 minutes, remove the supernatant, and resuspend the eggs in 5 milliliters of M9 buffer or sterile water [4]. **NOTE: The VO is edited for additional shots**
- 2.9.1. Talent washing the egg pellet with sterile distilled water.
- ~~2.9.2. Talent resuspending the eggs in 5 milliliters of M9 buffer or sterile water.~~
- 2.9.3. **Added shot: Talent resuspending the eggs in 5 mL 60 % sucrose and equal volume of sterile water.**
- 2.9.4. **Added shot: Talent collects the eggs from the sucrose gradient ring and washes them with sterile water. Talent places the tube in the centrifuge. After centrifugation, Talent removes the supernatant and resuspends the eggs in 5 mL of M9 buffer or sterile water.**
- 2.10. To prepare the egg stock solution, gently mix the egg suspension before sampling, as the eggs rapidly sediment [1].
- 2.10.1. Talent gently mixing the egg suspension.
- 2.11. Then place three 20-microliter drops on a slide [1], and count the eggs under a stereomicroscope [2].
- 2.11.1. Talent placing three 20-microliter drops on a slide.
- 2.11.2. SCOPE: 2.11.2-eggs.mp4: 00:39-00:43
- 2.12. ~~Calculate the average egg count [1] and~~ Adjust the concentration to 50 to 100 eggs per 100 microliters using M9 buffer [2]. Mix the egg suspension thoroughly before aliquoting to ensure uniformity [3]. Then, aliquot the required volume for each experimental condition as per the selected protocol [4]. **NOTE: The VO is edited for the deleted shot**
- ~~2.12.1. Talent calculating the average egg count.~~ **NOTE: Not filmed**

2.12.2. SCOPE: 2.12.2-eggs-adjusting.mp4: 00:18-00:25

2.12.3. Talent mixing the egg suspension again. NOTE: This step was filmed as a continuation of Step 2.11.1.

2.12.4. Talent aliquoting the egg suspension for an experimental condition.

3. Egg-Hatching Assay in Microcentrifuge Tubes

3.1. Prepare drug-free agar plates by adding 1.7 grams of agar to 100 milliliters of distilled water without adding nutrients or bacteria [1]. Pour approximately 3 milliliters of agar into each 35-millimeter Petri dish to maintain a consistent agar depth across experiments [2].

3.1.1. Talent preparing the agar solution with agar and distilled water.

3.1.2. Talent pouring approximately 3 milliliters of agar into a 35 millimeter Petri dish.

3.2. Prepare agar plates 12 to 24 hours before use to prevent excessive drying, which can reduce egg viability and affect larval movement [1].

3.2.1. Talent arranging freshly prepared agar plates for storage.

3.3. Divide each plate into four quadrants after solidification to facilitate counting [1]. Store the plates at 8 to 10 degrees Celsius in sealed conditions to prevent dehydration [2].

3.3.1. Talent marking a solidified agar plate into four quadrants.

3.3.2. Talent placing sealed agar plates into cold storage.

3.4. Next, mix the egg suspension gently [1] and transfer 500 microliters of the egg suspension into a 1.5 milliliter microcentrifuge tube for each condition [2].

3.4.1. Talent gently mixing the egg suspension.

3.4.2. Talent transferring 500 microliters of the egg suspension into a 1.5 milliliter microcentrifuge tube. NOTE: This step was filmed as a continuation of Step 3.4.1

3.5. Add 500 microliters of the treatment solution, trans-cinnamaldehyde and Linalyl Acetate, or the corresponding control, to the 500 microliters of egg suspension [1]. Incubate at 22 degrees Celsius for 4 to 8 hours [2].

3.5.1. Talent adding the treatment solution to the egg suspension.

3.5.2. Talent placing the microcentrifuge tube into the incubator. NOTE: This step was filmed as a continuation of Step 3.5.1

3.6. During incubation, maintain gentle agitation using a tube rotator or rocking platform to prevent egg sedimentation and ensure uniform exposure [1]. Avoid static conditions to prevent uneven drug exposure [2].

- 3.6.1. Talent placing the tubes on a tube rotator or rocking platform.
- 3.6.2. Shot of the tubes rotating uniformly throughout the incubation period.
- 3.7. Mark one side of each tube before centrifugation to facilitate rapid pellet localization and prevent accidental loss during supernatant removal [1]. Centrifuge the tubes at 3,420 *g* for 3 minutes at 18 to 25 degrees Celsius after incubation [2]. Identify the pellet as a small white deposit on the tube wall [3].
 - 3.7.1. Talent marking one side of the microcentrifuge tube before centrifugation.
 - 3.7.2. Talent placing the tubes in the centrifuge.
 - 3.7.3. Shot of the small white pellet visible on the wall of the microcentrifuge tube.
- 3.8. Remove the supernatant carefully without disturbing the pellet [1]. ~~Wash the eggs three times with M9 buffer to eliminate residual compound. Ensure thorough resuspension between washes [2].~~ Then, resuspend the final pellet in 240 microliters of M9 buffer [3]. NOTE: The VO is edited for the deleted shot
 - 3.8.1. Talent carefully removing the supernatant from the microcentrifuge tube.
 - 3.8.2. ~~Talent washing the egg pellet with M9 buffer.~~ NOTE: Not filmed
 - 3.8.3. Talent resuspending the final pellet in 240 microliters of M9 buffer.
- 3.9. Allow the plates to equilibrate to room temperature before use [1]. Plate the suspension onto the agar plates by dispensing 20 microliters into each quadrant. Use three plates for each condition [2]. Allow the drops to dry completely before moving or inverting the plates to prevent egg displacement across the surface [3]. Perform egg hatching on and multi-well plates [4] and drug-containing agar plates [5]. NOTE: The VO is adjusted for the additional shot
 - 3.9.1. Talent placing the plates on the bench to equilibrate to room temperature.
 - 3.9.2. Talent dispensing 20 microliters of the suspension into a quadrant of an agar plate.
 - 3.9.3. Shot of the plated drops drying on the agar surface before the plates are handled.
 - 3.9.4. Talent adding the eggs to drug-containing plates and multi-well plates.
 - 3.9.5. Added shot: Talent adding the eggs to drug-containing plates

4. Evaluation and Counting

Demonstrator: Alcibeth Pulido Carrasquero

- 4.1. Evaluate egg hatching 24 hours after egg isolation, ensuring that the control conditions have reached complete hatching under the defined temperature [1]. ~~Calculate this time point from the moment of egg collection to allow sufficient embryonic development~~

~~[2]. Consider any eggs that remain unhatched at this stage as affected by the treatment [3].~~ **NOTE: The VO is edited for the deleted shot**

4.1.1. SCOPE: 4.1.1-control-L1.mp4: 00:06-00:16

~~4.1.2. Talent calculating the observation time based on the egg collection point.~~ **NOTE: Not filmed**

4.1.3. SCOPE: 4.1.3-trated.mp4: 00:17-00:24

4.2. Examine the samples under a stereomicroscope [1]. ~~For each condition, quantify the number of unhatched eggs [2] and the number of hatched L1 larvae [3].~~ **NOTE: The VO is edited for the deleted shot**

4.2.1. Talent examining the samples under a stereomicroscope.

~~4.2.2. SCOPE: Unhatched eggs visible within the field of view and being counted.~~ **NOTE: Redundant step not filmed**

~~4.2.3. SCOPE: Hatched L1 larvae visible within the field of view and being counted.~~

4.3. Scan the entire observation area, including the plate edges and well boundaries [1]. Identify motile L1 larvae that may distribute unevenly and accumulate in these regions [2]. Finally, perform image acquisition and data analysis [3].

4.3.1. Talent scanning the entire observation area under the stereomicroscope.

4.3.2. SCOPE: 4.3.2.mp4: 00:15-00:25

4.3.3. Talent in front of the computer, looking through the acquired images.

Results

5. Results

5.1. Trans-cinnamaldehyde produced clear concentration-dependent inhibition of egg hatching for microcentrifuge tubes [1], direct exposure on agar plates [2], and multiwell plates [3].

5.1.1. LAB MEDIA: Figure 1A.

5.1.2. LAB MEDIA: Figure 1B.

5.1.3. LAB MEDIA: Figure 1C.

5.2. The estimated IC_{50} (*I-C-Fifty*) value was 0.19 millimolar in microcentrifuge tubes [1], 0.1758 millimolar on agar plates [2], and 0.2267 millimolar in multiwell plates [3].

5.2.1. LAB MEDIA: Figure 1A. *Video editor: Highlight the third point from above*

5.2.2. LAB MEDIA: Figure 1B. *Video editor: Highlight the 2nd point from above*

5.2.3. LAB MEDIA: Figure 1C. *Video editor: Highlight the third point from above*

1. Nematode

Pronunciation link: <https://www.merriam-webster.com/dictionary/nematode>

IPA: /'nem.əˌtoʊd/

Phonetic Spelling: nem·uh·tohd

2. Confluent

Pronunciation link: <https://www.merriam-webster.com/dictionary/confluent>

IPA: /'kɑːn.flu.ənt/

Phonetic Spelling: kon·floo·uhnt

3. Gravid

Pronunciation link: <https://www.merriam-webster.com/dictionary/gravid>

IPA: /'græv.id/

Phonetic Spelling: grav·id

4. Resuspend

Pronunciation link: <https://www.merriam-webster.com/dictionary/resuspend>

- IPA: /ˌriː.səˈspend/
Phonetic Spelling: ree·suh·spend
5. Agitate
Pronunciation link: <https://www.merriam-webster.com/dictionary/agitate>
IPA: /ˈædʒ.əˌteɪt/
Phonetic Spelling: aj·uh·tayt
 6. Incubation
Pronunciation link: <https://www.merriam-webster.com/dictionary/incubation>
IPA: /ˌɪn.kjəˈbeɪ.fən/
Phonetic Spelling: in·kyuh·bay·shuhn
 7. Viability
Pronunciation link: <https://www.merriam-webster.com/dictionary/viability>
IPA: /ˌvaɪ.əˈbɪl.ə.ti/
Phonetic Spelling: vy·uh·bil·uh·tee
 8. Sucrose
Pronunciation link: <https://www.merriam-webster.com/dictionary/sucrose>
IPA: /ˈsuː.kroʊs/
Phonetic Spelling: soo·krohs
 9. Gradient
Pronunciation link: <https://www.merriam-webster.com/dictionary/gradient>
IPA: /ˈɡreɪ.di.ənt/
Phonetic Spelling: gray·dee·uhnt
 10. Stereomicroscope
Pronunciation link: <https://www.merriam-webster.com/medical/stereomicroscope>
IPA: /ˌster.i.ooˈmaɪ.krəˌskoʊp/
Phonetic Spelling: stair·ee·oh·my·kruh·skohp
 11. Aliquot
Pronunciation link: <https://www.merriam-webster.com/dictionary/aliquot>
IPA: /ˈæl.i.kwɑːt/
Phonetic Spelling: al·ih·kwot
 12. Trans-cinnamaldehyde
Pronunciation link: <https://www.howtopronounce.com/trans-cinnamaldehyde>
IPA: /trænzˌsɪn.əˈmæl.dəˌhaɪd/
Phonetic Spelling: tranz sin·uh·mal·duh·hyde
 13. Linalyl
Pronunciation link: <https://www.howtopronounce.com/linalyl>
IPA: /lɪˈnæl.ɪl/
Phonetic Spelling: lih·nal·il
 14. Acetate
Pronunciation link: <https://www.merriam-webster.com/dictionary/acetate>
IPA: /ˈæs.əˌteɪt/
Phonetic Spelling: ass·uh·tayt
 15. Sedimentation
Pronunciation link: <https://www.merriam-webster.com/dictionary/sedimentation>

IPA: /ˌsed.ə.mənˈteɪ.ʃən/

Phonetic Spelling: sed·uh·mun·tay·shuhn

16. Equilibrate

Pronunciation link: <https://www.merriam-webster.com/dictionary/equilibrate>

IPA: /ˌiː.kwəˈlaɪ.breɪt/

Phonetic Spelling: ee·kwuh·ly·brayt

17. Embryonic

Pronunciation link: <https://www.merriam-webster.com/dictionary/embryonic>

IPA: /ˌem.briˈɑː.nɪk/

Phonetic Spelling: em·bree·on·ik

18. Larvae

Pronunciation link: <https://www.merriam-webster.com/dictionary/larva>

IPA: /ˈlɑːr.vi/

Phonetic Spelling: lar·vee

19. Concentration-dependent

Pronunciation link: <https://www.howtopronounce.com/concentration-dependent>

IPA: /ˌkɑːn.sənˈtreɪ.ʃən dɪˈpen.dənt/

Phonetic Spelling: kon·suhn·tray·shuhn dih·pen·duhnt

20. Microcentrifuge

Pronunciation link: <https://www.merriam-webster.com/dictionary/microcentrifuge>

IPA: /ˌmaɪ.kroʊˈsen.trə ˈfjuːdʒ/

Phonetic Spelling: my·kroh·sen·truh·fyooj