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Title: Using *Caenorhabditis elegans* to Screen for Tissue-Specific Chaperone Interactions

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Author Questionnaire

1. Microscopy: Does your protocol involve video microscopy, such as filming a complex dissection or microinjection technique? **N**

2. Software: Does the part of your protocol being filmed demonstrate software usage? **N**

3. Filming location: Will the filming need to take place in multiple locations (greater than walking distance)? **N**

Introduction

1. Introductory Interview Statements

REQUIRED:

1.1. **Anat Ben-Zvi**: The overall goal of the following experiments in the genetically tractable metazoan, *Caenorhabditis elegans*, is to identify tissue specific chaperone interactions **[1]**.

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

REQUIRED:

1.2. **Tomer Median**: The main advantage of this technique is that it combines easy to use assays, such as feeding RNAi and behavioral readouts, to expose novel, tissue-specific interactions **[1]**.

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

Protocol

2. Non-Stressful Embryo Synchronization

2.1. For a non-stressful embryo synchronization, use a worm pick to move about 100 eggs from an unsynchronized worm plate to a newly seeded NGM (N-G-M) plate **[1-TXT]** and cultivate the animals according to the appropriate experimental schedule **[2-TXT]**.

2.1.1. WIDE: Talent moving eggs **TEXT: NGM: nematode growth medium; See text for all medium and solution preparation details**

2.1.2. Talent placing plate into incubator/warm room **TEXT: *e.g.*, 15 °C: 5 d; 20 °C: 3.5 d; 25 °C: 2.5 d**

2.2. When the animals reach the first day of egg laying, slowly add 1 milliliter of M9 buffer to the plate distant to the bacterial lawn **[1]** and rotate the plate so that the buffer completely covers the plate **[2]**.

2.2.1. Buffer being added to plate

2.2.2. Plate being rotated

2.3. Tilt the plate to one side to remove the liquid **[1]** and wash the animals from the plate **[2-TXT]**.

2.3.1. Liquid being removed from tilted plate

2.3.2. Animals being washed from plate **TEXT: Repeat wash x3**

2.4. When all of the worms have been washed from the plate, use a standard plastic pipette tip to cut a square of agar around where the eggs are concentrated **[1-TXT]** and place the piece of agar onto a newly seeded NGM plate **[2]**.

2.4.1. Agar/eggs being cut **TEXT: Approximately 200 eggs required to produce enough egg-laying animals**

2.4.2. Agar being placed into plate

2.5. Cultivate the eggs under the same culture conditions **[1]**. At the end of the incubation, the plate should be covered with synchronized eggs **[2]**.

2.5.1. Talent placing plate into incubator/warm room

2.5.2. Shot of plate covered w/ synchronized eggs

2.6. After washing all of the animals from the plate as just demonstrated **[1]**, add 1 milliliter of fresh M9 buffer **[2]** and use a cell scraper to release the eggs from the plate **[3]**.

2.6.1. Talent washing plate *Videographer: Important step*

2.6.2. Talent adding buffer to plate, with buffer container visible in frame *Videographer: Important step*

2.6.3. Eggs being scraped *Videographer: Important step*

2.7. When all of the eggs have been detached, transfer the entire volume of buffer from the plate into a conical tube for centrifugation **[1-TXT]**. Centrifuge the tube **[2]** and discard the supernatant **[3]**.

2.7.1. Talent adding eggs to tube **TEXT: 2 min, 3000 x g, RT**

2.7.2. Talent placing samples in centrifuge.

2.7.3. Talent removing the supernatant.

NOTE: Two shots, 2.7.2 and 2.7.3, were added during the shoot in step 2.7, and VO description is added according to the added shots.

2.8. After removing the supernatant, add up to 1 milliliter of fresh M9 buffer to the plate **[1]** and pipette to resuspend the eggs **[2]**.

2.8.1. Talent adding buffer to a plate. Author's NOTE: Buffer was added in the plate, not in the tube.

2.8.2. Eggs being resuspended

2.9. Wash the eggs five more times as just demonstrated **[1]**. After the last wash, the egg pellet should appear white **[2-TXT]** and all but the last 200 microliters of supernatant can be removed **[3]**.

2.9.1. Talent placing tube(s) into centrifuge *Videographer: Difficult step*

2.9.2. Shot of pellet *Videographer: Difficult step* **TEXT: If pellet still yellow/brown, repeat wash until white**

2.9.3. Supernatant being removed *Videographer: Difficult step*

3. Common Phenotypic Assays: Animal Cultivation, Embryonic Lethality, and Paralysis

3.1. To cultivate synchronized nematodes for an experiment, place a drop of about 30 eggs close to the bacterial lawn in each interference RNA-seeded plate **[1]** and about 30 eggs onto a plate seeded with empty vector-containing bacteria **[2]**.

3.1.1. WIDE: Talent adding eggs to plate *Videographer: Difficult step*

3.1.2. Talent adding eggs to reference plate *Videographer: Difficult step*

3.2. Then cultivate the animals under the appropriate culture conditions according to the experimental protocol **[1]**.

3.2.1. Talent placing plate into incubator/warm room

3.3. To assess the embryonic lethality of the synchronized animals, when the animals begin to lay eggs, transfer about 100 eggs to an empty plate **[1]** and spread the eggs into rows to simplify the counting **[2]**.

3.3.1. Talent adding eggs to plate

3.3.2. Eggs being spread into rows

3.4. After 24-48 hours, score the percent of unhatched eggs **[1]**. For reference, compare the experimental animal eggs to the eggs of animals treated with empty vector-control bacteria **[2]**.

3.4.1. Shot of experimental plate after 24-48 hours, with experimental plate label *Videographer: Important step* visible in frame

3.4.2. Reference plate being placed next to experimental plate from 3.4.1., with reference plate label visible in frame *Videographer: Important step*

3.5. To set up a paralysis assay, cultivate age-synchronized animals on empty vector-control bacteria until the animals reach adulthood but before egg laying starts **[1]**.

3.5.1. Talent adding eggs to plate

3.6. Use a fine marker to draw a line on the back of a regular NGM agar plate **[1]** and place 5-10 animals on the marked line **[2]**.

3.6.1. Line being drawn

3.6.2. Animal(s) being placed

3.7. Set a timer for 10 minutes **[1]** and score the percentage of animals remaining on the line as paralyzed worms **[2]**.

3.7.1. Talent setting timer, with plate visible in frame *Videographer: Important step*

3.7.2. Shot of animals remaining on line, with wild type label visible in frame *Videographer: Important step*

3.8. For reference, compare this data to the percentage of mutant animals grown on empty vector-control bacteria **[1-TXT]**.

3.8.1. Plate with mutant animals being placed next to wild type plate, with mutant label visible in frame *Videographer: Important step* **TEXT: *e.g.*, wt animals treated with unc-45 RNAi exhibit severe paralysis and can be used as positive control**

Author's NOTE: Step 3.7-3.8: There were two images planed. The first (worms on a plate with a reference line) could not be taken because it is only visible via the scope. We can try to take it and send the image to you (this step is noted as 3.2.7A)
Image of plates with the indicated marking, we did take this image (3.8.1 in the protocol) - it is noted in filming as 3.7.2B.

4. Protein Knockdown Validation

4.1. For protein knockdown validation, place 250-300 synchronized eggs onto a 60-millimeter NGM-interference RNA plate seeded with the relevant double stranded RNA-expressing or empty vector-containing bacteria **[1]** and cultivate the eggs for the appropriate experimental period **[2]**.

4.1.1. WIDE: Talent adding eggs to plate

4.1.2. Talent placing plate into incubator/warm room

4.2. At the end of the incubation, transfer a total of 200 young adult animals into the cap of a 1.5-milliliter tube containing 200 microliters of PBS-T (P-B-S-T) maintained at the animals' cultivation temperature **[1-TXT]**.

4.2.1. Animals being added to cap, with plate and PBS-T container visible in frame
TEXT: PBS-T: PBS supplemented with 0.1% Tween-20

4.3. Close the cap carefully **[1]** and centrifuge the animals at 1000 x g for 1 minute **[2]**.

4.3.1. Cap being closed

4.3.2. Talent placing tube into centrifuge

4.4. At the end of the centrifugation, add 800 microliters of fresh PBS-T to the tube **[1]** and centrifuge the worms again **[2]**.

4.4.1. Talent adding PBS-T to tube, with PBS-T container visible in frame

4.4.2. Talent adding tube(s) to centrifuge

4.5. Carefully remove the top 900 microliters of the supernatant **[1]** and wash the nematodes in 800 microliters of fresh PBS-T three more times **[2]** as just demonstrated **[3]**.

4.5.1. Supernatant being removed

4.5.2. Talent adding PBS-T to tube, with PBS-T container visible in frame

4.5.3. Talent placing tube(s) in centrifuge.

NOTE: One shot 4.5.3 was added during the shoot in step 4.5, and the VO description was adjusted accordingly.

4.6. After the last wash, remove all but the last 100 microliters of the supernatant **[1]** and add 25 microliters of 5x sample buffer to the animals **[2]**.

4.6.1. Supernatant being removed

4.6.2. Sample buffer being added to tube, with sample buffer container visible in frame

4.7. Heat the samples for 10 minutes at 92°C and 1000 revolutions per minute **[1-TXT]** before loading 20 microliters of each sample onto an SDS-PAGE (S-D-S-page) gel **[2-TXT]**.

4.7.1. Talent placing sample at 92 °C with rotation **TEXT: Optional: Store samples at -20 °C**

4.7.2. Sample being loaded onto gel **TEXT: SDS-PAGE: sodium dodecyl sulfate-polyacrylamide gel**

4.8. Then perform western blot analysis using the appropriate antibodies to determine the relative stability of the protein of interest **[1]** and use densitometric software to determine the intensity of the bands **[2-TXT]**.

4.8.1. Talent adding antibody to blot

4.8.2. Talent at computer, analyzing bands **TEXT: Normalize experimental values to control samples**

Protocol Script Questions

A. Which steps from the protocol are the most important for viewers to see?
2.6., 3.4., 3.7., 3.8.

B. What is the single most difficult aspect of this procedure and what do you do to ensure success?
The protocol is very easy to perform and there are no real technical challenges. A sensitive step is 2.9 and the following 3.1, as carrying on OP50 bacteria and placing eggs on RNAi bacteria can results in contamination of RNAi and can thus lead to false negative results.

Results

5. Results: Representative Tissue-Specific Chaperone Interaction Screening

5.1. *Hsp-6* (H-S-P-six) knockdown during development results in a strong developmental arrest in wild type animals **[1]**.

5.1.1. LAB MEDIA: Figure 2A *Video Editor: please emphasize pink All Tissues bar*
5.1.2. LAB MEDIA: Figure 2B *Video Editor: please emphasize wild type images*

5.2. At the same time, *hsp-6* knockdown in a muscle-specific interference-RNA strain expressing wild type RDE-1 (R-D-E-one) in muscle tissue does not cause developmental arrest **[1]**.

5.2.1. LAB MEDIA: Figures 2A and 2B *Video Editor: please emphasize pink Muscle bar and Muscle images*

5.3. *Hsp-6* knockdown in an intestine-specific interference-RNA strain expressing wild type RDE-1 in intestinal cells, however results in a strong developmental arrest in phenotype **[1]**.

5.3.1. LAB MEDIA: Figures 2A and 2B *Video Editor: please emphasize pink Intestine bar and Intestine images*

5.4. A mutation in *hsp-6-mg585 (H-S-P-six-M-G-five-eighty-five)* that causes a mild growth delay can, therefore, be used to screen for aggravating or alleviating chaperone interactions by crossing a strain carrying that mutated gene into an intestinal-specific interference RNA strain and screening the chaperone interference RNA library **[1]**.

5.4.1. LAB MEDIA: Figure 2B *Video Editor: please emphasize D1 ahsa-1, sti-1, and daf-41 images*

5.5. MYO-3 (M-Y-O-three) organization in *unc-45-ts (unk-forty-five)* mutant animals treated with *sti-1 (S-T-eye-one)*, *ahsa-1 (A-H-S-A-one)* or *daf-41 (daff-forty-one)* interference RNA is similar to that of wild type worms at the fourth larval stage **[1]**, although the mutants exhibit disrupted sarcomeres after reaching adulthood **[2]**.

5.5.1. LAB MEDIA: Figure 3 *Video Editor: please emphasize pink sti-1, ahsa-1, and daf-41 data lines from L1-YA*
5.5.2. LAB MEDIA: Figure 3A *Video Editor: please emphasize pink sti-1, ahsa-1, and daf-41 data points at D1*

5.6. In contrast, both *unc-45-ts* mutant animals treated with an empty vector control **[1]** and wild type animals remain unaffected **[2]**.

5.6.1. LAB MEDIA: Figure 3A *Video Editor: please emphasize black and brown control data line from L1-D1*

5.6.2. LAB MEDIA: Figure 3A *Video Editor: please emphasize blue sti-1, ahsa-1, and daf-41 data lines from L1-D1*

Conclusion

6. Conclusion Interview Statements

6.1. **Anat Ben-Zvi**: Genetic interactions are not indicative of physical interactions. Thus, follow up experiments using genetic interaction screens must be performed to validate and directly examine the nature of the identified interactions **[1]**.

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