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Title: Culture and Assay of Large-Scale Mixed-Stage *Caenorhabditis elegans* Populations

Authors and Affiliations: Amanda O. Shaver[1], Goncalo J. Gouveia[2], Pamela S. Kirby[3], Erik C. Andersen[4], and Arthur S. Edison[1,2,3]

[1]Department of Genetics, University of Georgia
[2]Department of Biochemistry and Molecular Biology, University of Georgia
[3]Complex Carbohydrate Research Center, University of Georgia
[4]Department of Molecular Biosciences, Northwestern University

Corresponding Author:
Arthur S. Edison
aedison@uga.edu

Co-authors:
amanda.shaver@uga.edu
goncalog@uga.edu
pkirby@ccrc.uga.edu
erik.andersen@northwestern.edu

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? **Y**

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

Protocol Length
Number of Shots: **0**

Introduction

1. Introductory Interview Statements

REQUIRED:

1.1. **Amanda O. Shaver**: This technique allows us to collect large-scale worm populations to be used for multiple omics platforms with minimal manipulation **[1]**.

1.1.1. LAB MEDIA: To be provided by Authors: Named talent says the statement above in an interview-style shot, looking slightly off-camera

REQUIRED:

1.2. **Amanda O. Shaver**: This technique allows us to collect mixed-stage *C. elegans* populations across multiple omics experiments to obtain a holistic picture of each sample **[1]**.

1.2.1. LAB MEDIA: To be provided by Authors: Named talent says the statement above in an interview-style shot, looking slightly off-camera

Protocol

2. Large-Scale Culture Plate (LSCP) Gravid Adult Spot Bleaching

2.1. For spot bleaching of gravid adults onto large-scale culture plates, flame a worm pick over a Bunsen burner **[1]** and use the pick to scoop fresh *E. coli* from the edge of the bacterial lawn onto a large-scale culture plate **[2]**.

2.1.1. LAB MEDIA: To be provided by Authors: Talent flaming pick over lit burner
2.1.2. LAB MEDIA: To be provided by Authors: Bacteria being scooped, with LCSP visible in frame

2.2. Pick a single gravid adult from the fourth chunk plate for spot bleaching **[1]** and add 5 microliters of freshly prepared alkaline hypochlorite solution into one corner of the large-scale culture plate away from the *E. coli* lawn **[2-TXT]**.

2.2.1. LAB MEDIA: To be provided by Authors: Talent picking adult
2.2.2. LAB MEDIA: To be provided by Authors: Talent adding solution to plate **TEXT: See text for all solution preparation details**

2.3. Place the gravid adult into the alkaline hypochlorite solution **[1]** and tap the nematode to disrupt the cuticle and to release eggs **[2]**.

2.3.1. LAB MEDIA: To be provided by Authors: Adult being placed into solution *Videographer/Video Editor: Shot will be used again*
2.3.2. LAB MEDIA: To be provided by Authors: Adult being tapped

2.4. When a total of 5 adults have been placed evenly around the *E. coli* lawn in the same manner **[1]**, place the lid back onto the plate **[2]**.

2.4.1. LAB MEDIA: To be provided by Authors: 5th adult being placed
2.4.2. LAB MEDIA: To be provided by Authors: Talent placing lid onto plate

3. LSCP Sample Harvest

3.1. To harvest samples from large-scale culture plates, pour 50 milliliters of M9 solution onto one large-scale culture plate surface **[1]** and swirl the plate to ensure that the M9 covers the entire nematode growth medium agarose surface **[2]**.

3.1.1. LAB MEDIA: To be provided by Authors: Talent adding M9 solution to plate, with M9 container visible in frame

3.1.2. LAB MEDIA: To be provided by Authors: Talent swirling plate

3.2. Prime a sterile serological pipette with M9 **[1]** and tilt the plate so that the M9 and worm population gather in one corner of the plate **[2]**.

3.2.1. LAB MEDIA: To be provided by Authors: Pipette being primed
3.2.2. LAB MEDIA: To be provided by Authors: Talent tilting plate

3.3. Use the primed pipette to aspirate the worm suspension **[1]** and add the worms to a 50-milliliter conical tube **[2]**.

3.3.1. LAB MEDIA: To be provided by Authors: Worms being aspirated
3.3.2. LAB MEDIA: To be provided by Authors: Talent adding worms to tube

3.4. Place the tube on a rocker to disrupt any bacteria clumps and debris **[1]**.

3.4.1. LAB MEDIA: To be provided by Authors: Talent placing tube onto rocker

3.5. When all of the worms have been collected from all three plates **[1]**, transfer 15 milliliters of worms from each tube into each of three 15-milliliter conical tubes **[2]** and sediment the worms in the tubes by centrifugation **[3-TXT]**.

3.5.1. LAB MEDIA: To be provided by Authors: Tubes on rocker
3.5.2. LAB MEDIA: To be provided by Authors: Talent adding worms to tube
3.5.3. LAB MEDIA: To be provided by Authors: Talent placing tube(s) into centrifuge **TEXT: 1 min, 884 x g, 4 °C**

3.6. Carefully aspirate the supernatants without disturbing the worm pellets **[1]** and add 13 milliliters of worm suspension to each tube **[2]**.

3.6.1. LAB MEDIA: To be provided by Authors: Supernatant being aspirated
3.6.2. LAB MEDIA: To be provided by Authors: Talent adding worm suspension to tube

3.7. Invert the tubes to resuspend the pellets and to wash off as much bacteria and debris as possible **[1]** and centrifuge the worms again **[2]**.

3.7.1. LAB MEDIA: To be provided by Authors: Tube(s) being inverted
3.7.2. LAB MEDIA: To be provided by Authors: Tube(s) being placed into centrifuge

3.8. When each entire worm population has been collected, wash the worm pellets three times in 10 milliliters of fresh M9 solution per wash **[1]**.

3.8.1. LAB MEDIA: To be provided by Authors: Shot of pellet, then M9 solution being added to tube, with M9 solution container visible in frame

3.9. After the last wash, resuspend each cleaned wormed pellet in 10 milliliters of double distilled water **[1]**.

3.9.1. LAB MEDIA: To be provided by Authors: Talent adding water to tube

4. Population Size Estimation

4.1. For population size estimation, quickly dilute three 100-microliter aliquots of warm sample from each tube in 900 microliters of M9 solution per sample **[1-TXT]** and use the aliquots to make serial dilutions **[2-TXT]**.

4.1.1. LAB MEDIA: To be provided by Authors: Talent adding sample to 24-well plate, with M9 container visible in frame **TEXT: Determine worm numbers in <5 min**

4.1.2. LAB MEDIA: To be provided by Authors: Talent making dilution of one sample **TEXT: *i.e.*, 1:10, 1:100, 1:000**

4.2. Place the stock worm sample suspensions on a rocker for continuous movement of the cultures while the aliquots are being counted **[1]** and mix the worm dilutions until they are homogenous **[2]**.

4.2.1. LAB MEDIA: To be provided by Authors: Plate rocking on rocker

4.2.2. LAB MEDIA: To be provided by Authors: Talent mixing worm dilution

4.3. Add 5 microliters of solution from the first 1:10 worm sample to a glass microscope slide **[1]** and count the worms by light microscopy **[2]**.

4.3.1. LAB MEDIA: To be provided by Authors: Talent adding worms to slide, with plate visible in frame

4.3.2. LAB MEDIA: To be provided by Authors: Talent at microscope, counting worms

4.4. If there are less than 50 worms in the sample, count the 1:100 and 1:1000 dilutions **[1]**.

4.4.1. LAB MEDIA: To be provided by Authors: Talent adding worms to slide, plate visible in frame

4.5. If there are more than 50 worms, move to the next serial dilution **[1]**.

4.5.1. LAB MEDIA: To be provided by Authors: Talent selecting next dilution for counting

4.6. After counting each aliquot replicate of each dilution three times, average the dilution counts to determine the estimated population size of the worm **[1]**.

4.6.1. LAB MEDIA: To be provided by Authors: Talent calculating average on phone

4.7. Then split the worm samples into the appropriate experimental aliquots **[1]** and flash freeze the samples liquid nitrogen for minus 80-degree Celsius storage **[2]**.

4.7.1. LAB MEDIA: To be provided by Authors: Talent splitting worms into aliquots
4.7.2. LAB MEDIA: To be provided by Authors: Talent dipping sample into LN2

5. Large Particle Flow Cytometry (LPFC) Preparation

5.1. To prepare a worm sample for large particle flow cytometry, dilute an approximately 5 x 10^4 mixed stage worm aliquot to a final volume of 10 milliliters of M9 solution **[1]** and add 200 microliters of a 1-milligram/milliliter *E. coli* and 1:50 dilution of 0.5-micromolar red fluorescent microsphere solution to the worm suspension **[2]**.

5.1.1. LAB MEDIA: To be provided by Authors: Talent adding M9 to worms
5.1.2. LAB MEDIA: To be provided by Authors: Talent adding fluorescent microspheres to worms

5.2. After a 20-minute incubation at room temperature with rocking, collect the worms and microspheres by centrifugation **[1]** and wash the worms two times with fresh M9 solution to remove any excess bacteria and uninternalized microspheres **[2]**.

5.2.1. LAB MEDIA: To be provided by Authors: Talent placing tube(s) into centrifuge
5.2.2. LAB MEDIA: To be provided by Authors: Shot of pellet if visible, then M9 being added to tube, with M9 container visible in frame

5.3. After the second wash, resuspend the pellet in 5 milliliters of M9 solution **[1]**. If the pellet looks clean, add 5 milliliters of M9 supplemented with 50-millimolar sodium azide to the worms **[2]** and place the tube on a rocker to both straighten and euthanize the worms for accurate counting and sizing **[3]**.

5.3.1. LAB MEDIA: To be provided by Authors: Talent adding M9 to tube, with M9 container visible in frame
5.3.2. LAB MEDIA: To be provided by Authors: Shot of clean looking pellet, then M9 + sodium azide being added to tube, with M9 + sodium azide container visible in frame
5.3.3. LAB MEDIA: To be provided by Authors: Tube on rocker

6. Population Distribution Documentation and 384-Well Imaging Plate Preparation

6.1. For population distribution documentation, open the calibrated 384-well plate template **[1]** and set the template to dispense 20 gated objects into four wells to obtain four technical replicates of each gated region for each of the 20 bar regions of the sample **[2]**.

6.1.1. LAB MEDIA: To be provided by Authors: Talent opening template, with monitor visible in frame **TEXT: See text for full LPFC set up details**
6.1.2. SCREEN: To be provided by Authors: Template being set **TEXT: See supplement for sample worm dispensing layout**

6.2. Load a 40-milliliter sample of worms onto the large particle flow cytometer **[1]** and begin automatically sorting the sample **[2]** while continuously stirring the sample to prevent settling **[3]** and simultaneously dispensing objects from the sample into the calibrated 384-well plate **[4]**.

6.2.1. LAB MEDIA: To be provided by Authors: Talent loading sample onto cytometer
6.2.2. LAB MEDIA: To be provided by Authors: Talent starting sort, with monitor visible in frame
6.2.3. LAB MEDIA: To be provided by Authors: Sample being stirred
6.2.4. LAB MEDIA: To be provided by Authors: Objects being dispensed

6.3. Once entire sample has been sorted and the maximum number of gated regions have been dispensed into the 384 well plate, remove the sample from the cytometer **[1]** and clean the instrument **[2]**.

6.3.1. LAB MEDIA: To be provided by Authors: Talent removing plate
6.3.2. SCREEN: To be provided by Authors: Instrument being cleaned

Results

7. Results: Representative Large-Scale Mixed-Stage *C. elegans* Population Evaluation

7.1. The large-scale culture plate method was tested on 15 strains of *C. elegans* **[1]**, including a mixture of *Caenorhabditis* Genetics Center mutants **[2]** and *Caenorhabditis elegans* Natural Diversity Resource wild strains **[3]**.

7.1.1. LAB MEDIA: Supplementary Table 3
7.1.2. LAB MEDIA: Supplementary Table 3 *Video Editor: please emphasize CGC strains*
7.1.3. LAB MEDIA: Supplementary Table 3 *Video Editor: please emphasize CeNDR strains*

7.2. In this analysis, the large-scale culture plate method yielded population sizes **[1]** from approximately 94,500 **[2]** to 9,290,000 **[3]**.

7.2.1. LAB MEDIA: Figure 3
7.2.2. LAB MEDIA: Figure 3 *Video Editor: please emphasize AUM2073 data box*
7.2.3. LAB MEDIA: Figure 3 *Video Editor: please emphasize VC2524 and DL238 data boxes*

7.3. The mean population size within the reference strain, PD1074 (P-D-one-zero-seven-four), and across strains was approximately 2.4 million worms **[1]**.

7.3.1. LAB MEDIA: Figure 3 *Video Editor: please emphasize PD1074 data box*

7.4. No significant differences were found in estimated population sizes between *C. elegans* strains over the course of an average of 12.2 large-scale culture plate growth days **[1]**.

7.4.1. LAB MEDIA: Figure 4

7.5. PD1074 large-scale culture plates took between 10-14 days to grow to a full mixed-stage population **[1]** with a mean growth time of 12.2 days **[2]**.

7.5.1. LAB MEDIA: Figure 4 *Video Editor: please add dotted horizonal lines at 10 and 14 days*
7.5.2. LAB MEDIA: Figure 4 *Video Editor: please emphasize dotted horizontal line at 10 days*

7.6. The slowest growing strain grew for a maximum of 20 days **[1]** and the fastest growing strain grew for a minimum of 10 days **[2]**.

7.6.1. LAB MEDIA: Figure 4 *Video Editor: please emphasize VC2512 data bar*
7.6.2. LAB MEDIA: Figure 4 *Video Editor: please emphasize KJ550 data bar*

7.7. In this sample distribution for PD1074, worms were measured from the L1 stage through gravid adult on a large particle flow cytometer **[1]**.

7.7.1. LAB MEDIA: Figure 5

7.8. Subsequent imaging **[1]** and visualization of the variations in the population distribution across samples **[2]** revealed that the pipeline generated a mixed-stage population of *C. elegans* **[3]**.

7.8.1. LAB MEDIA: Figures 6 and 7 *Video Editor: please emphasize Figure 6*
7.8.2. LAB MEDIA: Figures 6 and 7 *Video Editor: please emphasize Figure 7*
7.8.3. LAB MEDIA: Figures 6 and 7

Conclusion

8. Conclusion Interview Statements

8.1. **Amanda O. Shaver**: It is important to accurately count the worms throughout the sample collection so that you obtain data that is comparable across growths and studies **[1]**.

8.1.1. LAB MEDIA: To be provided by Authors: Named talent says the statement above in an interview-style shot, looking slightly off-camera (4.1.-4.7.)

8.2. **Amanda O. Shaver**: This collection approach can be applied to RNA sequencing, genomics, and other omics analyses to expand our understanding of the complicated dynamics that occur within *C. elegans* populations **[1]**.

8.2.1. LAB MEDIA: To be provided by Authors: Named talent says the statement above in an interview-style shot, looking slightly off-camera

8.3. **Amanda O. Shaver**: This technique allows us to collect mixed-stage *C. elegans* populations across multiple omics experiments to obtain a holistic picture of each sample **[1]**.

8.3.1. LAB MEDIA: To be provided by Authors: Named talent says the statement above in an interview-style shot, looking slightly off-camera