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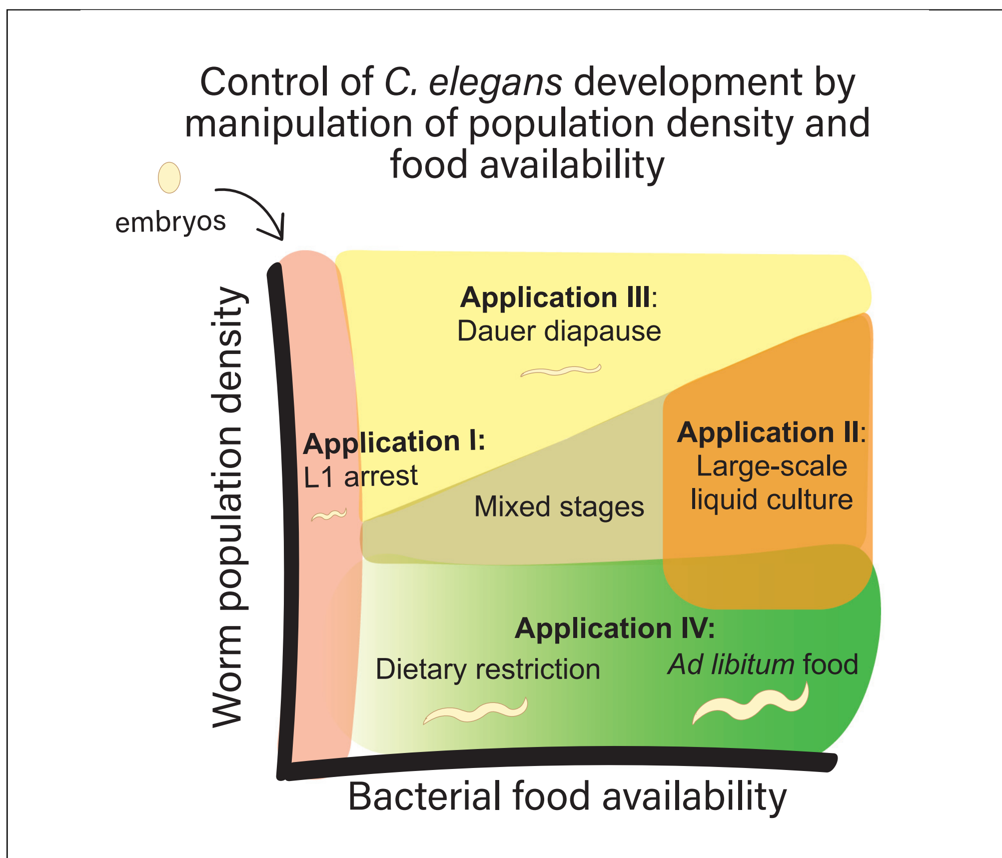
Protocol

Liquid-culture protocols for synchronous starvation, growth, dauer formation, and dietary restriction of *Caenorhabditis elegans*



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HIGHLIGHTS

A set of liquid-culture protocols for a variety of applications in *C. elegans*

Stringent starvation-induced developmental arrest of L1-stage larvae

Production of pure populations of dauer larvae without pheromone, mutants, or selection

Dietary restriction based on bacterial dilution rather than mutants

Hibshman et al., STAR Protocols 2, 100276
March 19, 2021 © 2020 The Authors.
https://doi.org/10.1016/j.xpro.2020.100276

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Liquid-culture protocols for synchronous starvation, growth, dauer formation, and dietary restriction of *Caenorhabditis elegans*

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https://doi.org/10.1016/j.xpro.2020.100276

SUMMARY

Standard laboratory culture of *Caenorhabditis elegans* utilizes solid growth media with a bacterial food source. However, this culture method limits control of food availability and worm population density, factors that impact many life-history traits. Here, we describe liquid-culture protocols for precisely modulating bacterial food availability and population density, facilitating reliable production of arrested L1 larvae, dauer larvae, dietarily restricted worms, or well-fed worms. Worms can be grown in small quantities for standard assays or in the millions for other applications.

For complete details on the use and execution of these protocols, please refer to Hibshman et al. (2016), Webster et al. (2018), and Jordan et al. (2019).

BEFORE YOU BEGIN

Many aspects of culturing *C. elegans* in the lab have been well defined and are described elsewhere (Lewis and Fleming, 1995; Stiernagle, 2006). Here, we provide methods for liquid culture of *C. elegans* to produce homogeneous, synchronized cultures in defined conditions including starvation (L1 arrest), well-fed, dauer-forming, and dietary restriction (Table 1). These methods each begin with a commonly used bleaching protocol to produce a sterile, relatively synchronous population of embryos, allowing liquid cultures with variable worm and bacterial densities to be established. These methods will be of use to researchers seeking controlled culture conditions to consistently produce specific developmental outcomes.

Preparation of bacterial food source

⏲ Timing: 3 days

In order to establish liquid cultures, several components must be prepared ahead of time. S-complete should be prepared according to the recipe below, and a concentrated stock of bacteria should be prepared as food for the worms (Lewis and Fleming, 1995; Stiernagle, 2006).

1. Set up starter culture of bacteria
   a. Streak *E. coli* HB101 from a frozen stock onto a plate with LB agar with streptomycin selection. Grow overnight at 37°C.

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Table 1. Summary of bacterial and worm concentrations for different liquid culture applications

Bacterial concentration	Worm concentration	Application
50 mg/mL	1–5/μL	Large-scale liquid culture
1 mg/mL	5/μL	Dauer formation
0 mg/mL	1/μL	Starvation, L1 arrest
25 mg/mL	1/100μL	*Ad libitum* feeding
3.1 mg/mL	1/100μL	Dietary restriction

"1×" bacterial concentration refers to 25 mg/mL (OD_{600} ≈ 16).

   b. Using a wire loop or disposable plastic pipette tip, inoculate a single colony of HB101 into culture of 5 mL of LB with 50 μg/mL streptomycin in a glass test tube or similar tube.
   c. Grow overnight at 37°C with shaking.
2. Set up large culture
   a. Add the 5 mL starter culture of HB101 to 1 L of TB with streptomycin to a ~3 L flask.
   b. Grow for 24 h at 37°C at 250 rpm to allow bacteria to enter stationary phase.
3. Harvest bacteria
   a. Measure the mass of 250 mL plastic centrifuge bottles.
   b. Add overnight bacterial cultures to the bottles and spin at 5,000 rpm (4,000 × *g*) in a large centrifuge for 10 min at 21°C–22°C.
   c. Aspirate the supernatant and determine the mass of bacteria in tubes by subtracting the mass of the bottle alone from the mass of the bottle with bacteria.
   d. Resuspend the bacteria in a volume of S-complete equal to 4× the mass of bacteria to achieve a suspension of 250 mg/mL and vortex to mix. This is considered to be a 10× stock. The 1× concentration should be equivalent to OD_{600} ≈ 16.
4. Store concentrated stock bacteria at 4°C.

⚠ CRITICAL: Bacterial stocks can be stored at 4°C for approximately 1 month.

Note: HB101 is used for liquid culture rather than other strains of *E. coli* since it clumps less, but strains such as OP50 or HT115 could potentially be used if necessary.

KEY RESOURCES TABLE

REAGENT or RESOURCE	SOURCE	IDENTIFIER
Bacterial and virus strains		
HB101	CGC	N/A
Chemicals, peptides, and recombinant proteins		
Sodium hypochlorite solution	Sigma-Aldrich	239305-500 mL
NaCl	Fisher	S271-3 kg
$K_2HPO_4 \cdot 3H_2O$	Macron	7088-04-500 g
KH_2PO_4	Acros Organics	205920025-2.5 kg
Disodium EDTA	Sigma	E5134-1 kg
$FeSO_4 \cdot 7H_2O$	Sigma	F8633-250 g
$MnCl_2 \cdot 4H_2O$	Sigma	M8054-100 g
$ZnSO_4 \cdot 7H_2O$	Sigma	Z4750-100 g
$CuSO_4 \cdot 5H_2O$	Sigma-Aldrich	209198-100 g
Citric acid monohydrate	Sigma	C1909-500 g
Tri-potassium citrate monohydrate	Sigma-Aldrich	P1722-500 g
$CaCl_2$	Avantor	1311-01-500 g
$MgSO_4$	Macron	6070-12-500 g
Tryptone	BD	211705-500 g

(Continued on next page)

Continued		
REAGENT or RESOURCE	SOURCE	IDENTIFIER
Yeast extract	Apex	20-254-500 g
Glycerol	Sigma	G5516-1 L
KOH	Sigma-Aldrich	P1767-1 kg
Streptomycin	Sigma	S1567-25 g
Cholesterol	Sigma	C8667-25 g
Experimental models: organisms/strains		
N2	CGC	N/A
Other		
15 mL conical tubes	Corning	430791
25 mL flasks	Pyrex	4980-25 mL
500 mL flasks	Pyrex	5100
2,800 mL flasks	Pyrex	4420
16 mm test tubes	Pyrex	7082016XX
250 mL plastic centrifuge bottles	Nalgene	3141-0250

MATERIALS AND EQUIPMENT

Liquid cultures are maintained in either glass test tubes (2 mL in a 10 mm tube, 5 mL in a 16 mm tube, or 10 mL in a 25 mm tube) on a tissue culture roller drum (ideally in an incubator) or Erlenmeyer flasks (10%–20% of the flask volume; e.g., 10–20 mL in a 100 mL flask) in a shaker incubator. We have found that cultures are not well mixed when maintained in upright or slanted test tubes in a shaker, so this is avoided. In addition, a vortex, clinical centrifuge, stereomicroscope, and various pipets are required.

The following buffers are required for this protocol (see also Lewis and Fleming, 1995; Stiernagle, 2006):

S-Basal	
Reagent	Amount
NaCl	5.9 g
1 M KH_2PO_4, pH 6.0	50 mL
ddH_2O	to 1 L
Total	**1 L**

Adjust the pH of 1 M KH_2PO_4 to reach 6.0 using concentrated KOH (Lewis and Fleming, 1995). Autoclave to sterilize. Add 1 mL of cholesterol solution (5 mg/mL in 100% ethanol) after the solution has cooled (Lewis and Fleming, 1995; Stiernagle, 2006). Note, when using S-basal for starvation the cholesterol may or may not be added to media. Store at 21°C–22°C for up to approximately 1 year.

Trace metals solution	
Reagent	Amount
Disodium EDTA	1.86 g
$FeSO_4 \cdot 7H_2O$	0.69 g
$MnCl_2 \cdot 4H_2O$	0.2 g
$ZnSO_4 \cdot 7H_2O$	0.29 g
$CuSO_4 \cdot 5H_2O$	0.025 g
ddH_2O	to 1 L
Total	**1 L**

Sterilize by autoclaving and store in the dark (Lewis and Fleming, 1995; Stiernagle, 2006). We recommend wrapping bottles in foil. Store at 21°C–22°C for up to approximately 1 year.

1 M potassium citrate pH 6.0	
Reagent	**Amount**
Citric acid monohydrate	20 g
Tri-potassium citrate monohydrate	293.5 g
ddH_2O	to 1 L
Total	**1 L**

Adjust pH to 6.0 with concentrated KOH. Autoclave to sterilize (Lewis and Fleming, 1995; Stiernagle, 2006). Store at 21°C–22°C for up to approximately 1 year.

S-complete (or S-medium)	
Reagent	**Amount**
S-basal	1 L
1 M Potassium citrate pH 6.0	10 mL
Trace metals solution	10 mL
1 M $CaCl_2$	3 mL
1 M $MgSO_4$	3 mL
Total	**1,026 mL**

Add the additional components to S-basal using sterile technique (Lewis and Fleming, 1995; Stiernagle, 2006). Store at 21°C–22°C for up to approximately 1 month.

LB	
Reagent	**Amount**
Tryptone	10 g
NaCl	10 g
Yeast extract	5 g
ddH_2O	950 mL
Total	**1 L**

Adjust the pH to 7.0 and autoclave on a liquid cycle to sterilize (CSH, 2006). Store at 21°C–22°C for up to approximately 1 year.

TB	
Reagent	**Amount**
Tryptone (100 μM)	20 g
Yeast extract (100 μM)	24 g
Glycerol	4 mL
ddH_2O	to 900 mL
Phosphate buffer (0.17 M KH_2PO_4, 0.72 M K_2HPO_4)	100 mL
Total	**1 L**

Combine tryptone, yeast extract, glycerol, and ddH_2O. Autoclave on a liquid cycle to sterilize. After the buffer has cooled add 100 mL of sterile phosphate buffer (CSH, 2015). Store at 21°C–22°C for up to approximately 1 year.

Bleach solution	
Reagent	Amount
Hypochlorite	2 mL
5 M KOH or NaOH	1 mL
ddH_2O	7 mL
Total	10 mL

⚠ CRITICAL: Prepare bleach solution fresh for each use. Sodium hypochlorite and potassium hydroxide are corrosive and contact with skin and eyes should be avoided. Precautions should be taken when handling these chemicals, including wearing protective eyewear, a lab coat, and gloves. See the Safety Data Sheets for these chemicals for additional details. Use immediately.

STEP-BY-STEP METHOD DETAILS

Collect *C. elegans* embryos to add to cultures

Timing: ~1 h

In this step embryos will be isolated by hypochlorite treatment ("bleaching") to obtain a synchronous population at a defined density. Additional bleach protocols are described elsewhere (Lewis and Fleming, 1995; Porta-de-la-Riva et al., 2012; Stiernagle, 2006).

1. Prepare bleach solution.
   a. Determine how much bleach solution will be needed based on the number of strains that will be bleached. It is generally recommended to prepare at least 10 mL of bleach solution for each strain or condition that will be bleached separately.
   b. Prepare an appropriate amount of bleach solution: 1 part 5 M KOH or NaOH, 2 parts sodium hypochlorite solution (Sigma), and 7 parts ddH_2O.
2. Wash worms from plates or from liquid culture with many well-fed gravid adults.
   a. Wash worms from plates two times using ~3 mL of S-complete each time per 6 cm plate and aspirate with a 10 mL serological pipet into a 15 mL conical tube. After washing, plates can be observed under a microscope to ensure adequate collection of worms.
   b. Spin the worms at 3,000 rpm (1,500 × *g*) in a clinical centrifuge for 1 min and aspirate supernatant.
3. Add 3–4 mL bleach solution to the worms in the 15 mL conical tube. Start a stopwatch. Vortex constantly with periodic shaking of the tube to disrupt the worms.
4. After 1–2 min, spin the conical tube with worms at 3,000 rpm for 1 min to pellet.
5. Aspirate the bleach solution and add a fresh 3–4 mL of bleach solution to the tube. Shake and vortex the tube constantly until all worms have dissolved and only embryos are visible in the tube under a microscope. This may take up to 5 min more (7 min total time in bleach, including the centrifugation).
6. When only embryos are visible, pellet embryos by centrifuging at 3,000 rpm for 1 min. If dauers are present, they will either take longer to dissolve or will not dissolve completely.
   Troubleshooting 1
7. Wash the embryos 3× in 10 mL S-basal by centrifuging and aspirating while being careful to leave the pellet of embryos intact with ~100 μL of supernatant.
8. Resuspend the embryos in 10 mL of S-basal.
9. Determine and adjust the concentration of embryos.
   a. Vortex gently for a few seconds to evenly distribute the embryos, and take at least three 10–100 μL samples from the resuspended embryos and count the number of embryos in each sample. Increase sample size, or dilute the sample, so that at least 100–200 embryos in total are counted across all samples taken to ensure accurate determination of density. If

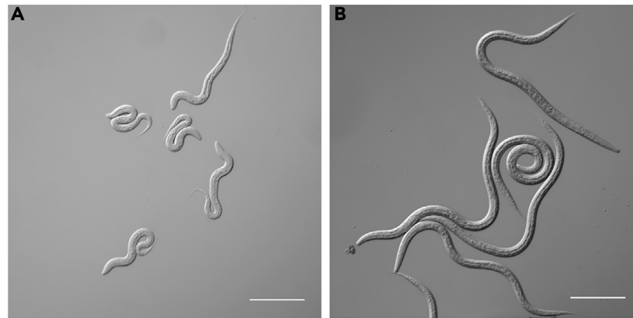

Figure 1. Representative images of *C. elegans* arrested as L1 larvae and dauer larvae resulting from liquid-culture growth in Applications I and III
(A) Starved L1 larvae.
(B) Dauer larvae. Scale bars, 50 μm.

necessary, spin the sample down at 3,000 rpm and aspirate the S-basal down to a lower volume to increase the concentration of embryos. Minimize pipetting of the embryos since they may stick to plastic pipettes.

b. Adjust the volume of S-basal such that embryos are at a concentration of 1/μL (or another desired concentration).
Troubleshooting 2

10. Embryos can be cultured on a roller drum or in an Erlenmeyer flask in a shaker to hatch (hatching occurs 10–14 h post-bleach) and arrest as L1 larvae (Application I), or they can be added to S-complete with HB101 for growth in rich, dauer-forming, or dietary-restriction conditions.

⚠ CRITICAL: Do not leave the worms and embryos in the bleach solution for too long. In most cases, embryos should be washed with S-basal by 10 min at the very most after bleach addition, ideally closer to 7 min. The shells of embryos protect them from the damaging effects of bleach, but they will also be compromised if left in the bleach for too long. As soon as worm carcasses have dissolved S-basal should be added to dilute the bleach solution and prevent damage to the embryos.

Note: Typically about ten embryos are recovered from each gravid worm bleached. Embryo yield therefore depends on the number of gravid worms per plate used. For example, a 10 cm plate seeded with a chunk from a fresh starved plate with many young larvae yields approximately 5,000 - 10,000 embryos when bleached on the first or second day of egg laying but before starving, and a similar 6 cm plate yields approximately one-third as many embryos. Alternatively, starvation can be avoided by either chunking plates before they starve, resulting in mixed-stage populations with a lower proportion of gravid adults and reduced embryo yield, or by using L4 larvae to seed plates and bleaching on the first or second day of progeny egg laying. For the latter, we typically use seven L4s per 10 cm plate (more could be used, but at this low density they avoid dietary restriction as progeny become gravid), typically yielding 2,000 - 5,000 embryos per plate when bleached after 96 h at 20°C. In summary, the number of gravid worms per plate at the time of bleaching can be estimated as an indication of embryo yield, and it is best to establish consistent culture methods for consistent yields.

Application I: starvation and L1 larval arrest

⏲ Timing: 15 min setup, 12 h to several weeks culture time

11. To starve worms, suspend embryos obtained from bleach at a concentration of 1/μL in S-basal. Embryos will hatch within approximately 12 h and enter L1 arrest (Figure 1A).

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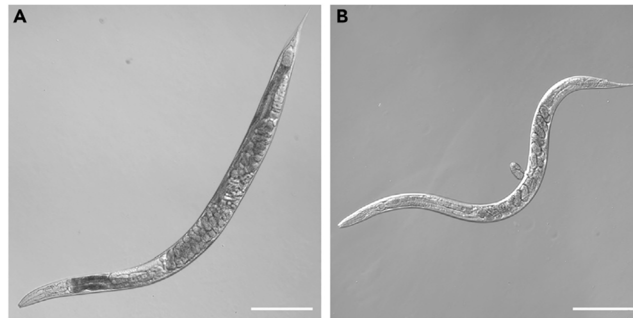

Figure 2. Representative images of *C. elegans* fed *ad libitum* or restricted diets in Application IV
(A) Adult worm after 96 h in *ad libitum* culture (25 mg/mL HB101).
(B) Adult worm after 96 h in dietary restriction (3.1 mg/mL HB101). Scale bars, 200 μm.

12. Incubate cultures in glass test tubes on a roller drum or in Erlenmeyer flasks at 20°C with shaking at 180 rpm. We recommend using a volume of 5 mL (5,000 total embryos) in a 16 mm tube, 10 mL (10,000 embryos) in a 25 mm tube, or 2 mL (2,000 embryos) in a 10 mm tube. For Erlenmeyer flasks, we recommend a volume equal to 10%–20% of the stated volume of the flask.

⚠ CRITICAL: Concentrations of worms other than 1/μL may be used; however, the density of larvae impacts starvation survival so consistency is essential (Artyukhin et al., 2013).

Note: As far as we know, S-basal without cholesterol or ethanol (virgin) is comparable to M9. S-basal can be used with or without cholesterol and ethanol, but the presence of ethanol approximately doubles the duration of starvation survival while maintaining developmental arrest (Castro et al., 2012).

Application II: Large-scale liquid culture in rich conditions

⏲ Timing: 15 min setup

Liquid cultures can be used to grow large quantities of synchronized worms.

13. A large-scale liquid culture can be established by diluting stock bacterial food to 50 mg/mL (2×) in S-complete and adding up to 5 embryos per μL (dauer formation occurs at higher densities). Worms can also be cultured at 1 per μL in 1× HB101. The culture is synchronized by starting with embryos, and tighter synchrony can be achieved by instead starting with larvae on the first day of L1 arrest (see Application I) (Baugh, 2009).
14. Incubate cultures at 20°C with shaking at 180 rpm for desired amount of time. For example, starting with embryos from bleach, cultures will be fully gravid after 72 h, and starting with arrested L1 larvae the first eggs are evident at 53 h and the cultures are fully gravid after 60 h. The worm and bacteria densities provided in step 3 are sufficient for the cultures to become fully gravid without starvation. We typically do not allow the cultures to continue after becoming fully gravid since *C. elegans* hold their eggs in liquid (Figure 2A).
15. Worms may be collected from liquid cultures by centrifugation in a clinical centrifuge (3,000 rpm or 1,500 × *g* for 1 min) in 15, 50, or 250 mL conical tubes. Large populations of worms also may be bleached for collection of large numbers of embryos to scale-up for different applications.
    Troubleshooting 3
    Troubleshooting 4
    Troubleshooting 5

Optional: Instead of starting liquid cultures with embryos collected from bleach treatment, it is also possible to wash worms from nematode growth media (NGM) plates and add them to liquid cultures. For example, a starved 6 cm plate can be used to start a 25 mL culture with 50 mg/mL (2×) HB101, producing a gravid population in 2–3 days. Also, if cultures begin to deplete the bacteria initially provided, additional bacteria can simply be added over time until the point of harvest (exchanging the buffer is not necessary), as long as bacterial density does not exceed 50 mg/mL (2×), but it is best to avoid this scenario.

Note: HB101 has no detectable proliferation in S-complete based on optical density, but bacterial density does decrease over time in these batch cultures due to consumption, potentially exposing worms to dietary restriction before collection (Hibshman et al., 2016). Worm density can be reduced to avoid this. If the density of worms in culture is too high, dauer larvae may begin to form even with high bacterial density. It is important to monitor cultures to ensure that worms are not entering the dauer state.

Application III: Dauer formation cultures

Timing: 15 min setup, 1 week to 1–2 months culture time

16. To establish a liquid culture that will cause worms to enter dauer diapause, suspend embryos following bleach or arrested L1 larvae at a density of 5/μL in S-complete and add bacterial HB101 food stock to 1 mg/mL.
17. Incubate the culture in an Erlenmeyer flask at 20°C with shaking at 180 rpm. We recommend using a 25 mL flask with a 5 mL volume of liquid culture, or a larger volume in larger flasks without exceeding 10%–20% of the flask's volume. We have not used glass test tubes in this application, but we presume they work as well.
18. Larvae develop slowly and enter dauer diapause by day 4 or 5 (Figure 1B). Dauers can be maintained for at least 40 days in culture while maintaining viability and the ability to recover.
19. Dauers can be recovered on NGM plates or in a new liquid culture (after brief washing) with reduced density and high food availability (see rich conditions described in Application II).

CRITICAL: The density and food concentration are critical for ensuring the vast majority of worms enter dauer diapause. Increasing food concentration or lowering worm population density will increase the frequency of worms that do not enter dauer diapause.

Note: There are several ways of identifying dauers as well as alternative protocols for dauer formation (Karp, 2018; Nika et al., 2016). Strengths of this protocol include that it works with wild-type worms, dauer pheromone is not necessary, SDS or other selection is not necessary, dauer formation is relatively synchronous, and culture parameters are reproducible.

Application IV: Dietary restriction

Timing: 15 min setup, 12 h to 4 days culture time

20. To set up conditions for dietary restriction, any dilution of food may be established to create the desired level of dietary restriction. We recommend assaying the phenotype of interest over a range of bacterial densities for optimization. We suggest using 25 mg/mL (1×) HB101 as a baseline well-fed (*ad libitum)* control. Based on maximum embryo size, we found 3.1 mg/mL HB101 (an 8-fold dilution of the 1×, 25 mg/mL, *ad libitum* concentration) to be optimal for dietary restriction (Hibshman et al., 2016). Embryos or L1 larvae should be added to a concentration of 1/100 μL such that bacterial density is not significantly depleted as worms develop, approximating a chemostat rather than a typical batch culture (Hibshman et al., 2016). Low worm density makes it more challenging to obtain extremely large populations in this application

compared to the others, but if this is limiting then density can be raised as long as there is no detectable effect on measured bacterial density over time during incubation.

21. Incubate cultures in Erlenmeyer flasks at 20°C with shaking at 180 rpm.
22. Worms can be collected for analysis at any point during their life cycle, or at 96 h for bleaching to collect their progeny (Figure 2).

EXPECTED OUTCOMES

The protocols included here describe methods to culture populations of worms that can be used for a variety of applications simply by varying the density of worms and bacteria. Methods for L1 larval arrest and starvation as well as dauer cultures reproducibly generate synchronized populations of *C. elegans* at defined developmental stages (Figure 1). These culture methods do not require sorting, selecting, or staging worms, and they do not require specific strains or reagents. Liquid culture methods of dietary restriction allow for careful titration of food availability. This is essential for studies that seek to define an optimum trait value across a range of food availability, as is advisable for analysis of genetic epistasis (Mair et al., 2009). Liquid-culture dietary restriction facilitates this type of analysis whereas other models of dietary restriction like *eat-2* mutants (with reduced pharyngeal pumping) do not. Furthermore, culture conditions can easily be scaled up for applications requiring millions of worms. Overall, these simple protocols should allow for production of large populations of worms at defined developmental stages cultured in well-controlled conditions, thereby facilitating a wide range of downstream applications.

LIMITATIONS

While liquid culture methods are highly effective for carefully regulating bacterial food availability and worm density, the physiology of worms in liquid is very different from those on solid media. Transcriptional and phenotypic differences have been documented, and liquid cultures are in some cases used as a model for exercising worms (Çelen et al., 2018; Laranjeiro et al., 2017). Notably, the shaking of cultures for these protocols likely limits the need for sustained swimming by the worms. However, the profound differences in physiology must be noted before designing experiments utilizing liquid culture. For example, hermaphrodites in liquid culture will hold their embryos and become bloated (Figure 2A), eventually dying from internal hatching, making studies of the germline and reproduction challenging after early adulthood. It is essential to only compare worms grown in liquid culture to control groups also grown in liquid culture in order to control for these physiological changes.

TROUBLESHOOTING

Problem 1

Worms are not dissolving in bleach solution.

Potential solution

It is essential to use fresh, high-quality bleach. Hypochlorite should be stored at 4°C and a fresh bleach solution (hypochlorite, KOH, and water) should be made each time immediately before harvesting embryos. It is also necessary to order fresh hypochlorite periodically as it can go bad over time. Once a bottle of hypochlorite solution is opened, we typically use it within about 2 months.

Problem 2

The yield of embryos from bleaching is very low.

Potential solution

One possible problem is that there were simply not enough gravid adults used for the protocol. We recommend picking ten L4 worms to a 10 cm plastic dish (with NGM and seeded with OP50) four days at 20°C in advance of the day of anticipated embryo collection. We expect a bleach yield of roughly ten embryos per gravid adult. Thus, collecting gravid adults from several such plates should

provide a sufficient yield for experiments requiring several thousand embryos. Alternatively, a chunk can be taken from a clean, freshly starved plate with many arrested larvae to a new plate and cultured for three-four days at 20°C prior to embryo collection. This approach involves the use of previously starved worms, but the plate will contain a higher proportion of gravid adults at the time of collection since the culture is more synchronous. Another possibility is that washing the worms from the plates was inefficient. Monitoring the effectiveness of the wash by looking at plates under a microscope before and after washing should indicate if this is the problem. A third possibility is that the embryos were left in the bleach too long and some embryos have degraded. Carefully monitor the degradation of worm carcasses and begin the wash steps immediately after no more worms are visible. It is possible that this may occur before the suggested 7 min total time in bleach. A fourth possibility is that embryos were lost during centrifugation and aspiration. Monitor the supernatant under a microscope to ensure it is relatively free of embryos and leave a larger volume of supernatant when aspirating, or use a serological pipet for aspiration rather than vacuum, if you believe this may be the case.

Problem 3

There are salt crystals in liquid cultures.

Potential solution

Occasionally there are crystals that precipitate in the liquid cultures. If crystals are observed in culture, it is best to re-make S-complete and prepare a fresh stock of concentrated bacteria. To assess cultures for the presence of crystals, a sample of the liquid culture can be plated on a 6 cm NGM plate. Observation of the sample under a microscope will indicate if any crystals are present.

Problem 4:

Liquid cultures have fungal or bacterial contamination.

Potential solution

To check for contamination, a sample of liquid culture can be plated on a 6 cm NGM plate. Growth beyond a uniform lawn of bacteria on the plate indicates contaminated cultures. If cultures become contaminated, they should be bleached and discarded, and new cultures should be established using sterile technique.

Problem 5

Cultures are not as synchronous as expected or developmental timing and staging vary between replicates.

Potential solution

This is likely due to an inconsistent preparation of embryos. The distribution of embryonic stages in the population of embryos prepared by bleaching influences the synchrony of cultures and the time it takes to reach developmental milestones. This distribution is affected by the age and feeding state of the worms used for bleaching. Older, stressed, and starved worms tend to hold their eggs, and bleaching them produces a population of embryos that spans a wider developmental range given older embryos. In addition, embryos should not be washed from the plate along with gravid worms for bleaching, since this will include older embryos. Instead, gravid worms should be gently washed from the plates, leaving embryos that have been laid stuck to the surface. Likewise, bleaching worms from liquid culture will include eggs already laid (this can be done reliably on the first day of egg laying with consistent culture conditions). Synchrony can be tightened by culturing embryos without food for 24 h so they hatch and enter L1 arrest (see Application I), and these arrested L1 larvae can be used to start liquid cultures (Applications II, III, and IV). However, for the best results, the culture conditions and history of the worms used for bleach should be as consistent as possible, which will result in populations of embryos with consistent stage distributions.

RESOURCE AVAILABILITY

Lead contact

Further information and requests for resources and reagents should be directed to and will be fulfilled by the Lead Contact, L. Ryan Baugh, ryan.baugh@duke.edu.

Materials availability

This study did not generate new unique reagents.

Data and code availability

This study did not generate new datasets.

ACKNOWLEDGMENTS

We thank Rojin Chitrakar for help in assembly of the key resources table. J.D.H. is supported by the National Institutes of Health (F32 GM131577). A.K.W. was supported by the NSF Graduate Research Fellowship Program. Work in the Baugh lab is funded by the National Institutes of Health (R01GM117408, L.R.B.).

AUTHOR CONTRIBUTIONS

L.R.B., J.D.H., and A.K.W. developed the protocols. J.D.H. and A.K.W. wrote the protocol and prepared the figures. All authors edited and approved the protocol.

DECLARATION OF INTERESTS

The authors declare no competing interests.

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