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*last updated: 10.01.25 (how to identify males and updated reagents)*

## Prerequisites

Basics of worm maintenance.

## Concepts

We discard plates contaminated with mold or bacterial growth (other than OP-50). These typically make for unhealthy worms. We also discard plates where the worms have burrowed into the agar. Loose pieces of agar and agarose in your sample can interfere with downstream reactions and sequencing.

Older plates (> 6 days) are more likely to have contamination and burrowing. For this protocol, worms need to be grown for about 8 days. On about day 6, pass the adults to new plates.

Bacteria in your sample will eat up reads, that is, DNA from bacteria will be sequenced in place of the nematode. We separate bacteria from nematodes by letting the nematodes settle in 15 mL conical tubes. Bacteria will stay in the supernatant and be discarded. Larvae do not like to settle in 50 mL conical tubes. Use 15 mL conical tubes when you can, and transfer to 15 mL prior to pelleting when you can't.

A higher occurrence of males is induced under starved conditions. Keep your plates fed with concentrated HB101. See Appendix A for identifying males (and young adult hermaphrodites).

## Notes

This protocol is based on development at 20 °C.

## Seeding bacteria

*E. coli*, OP-50 ( $OD_{600} > 0.6$ )

*E. coli*, HB101, concentrated

## Plates

6 cm NGM plates seeded with OP-50

10 cm NGM plates with 1.0 % agar and 0.7 % agarose (herein 10 cm NGM plates) seeded with OP-50

## Reagents and equipment

Eppendorf tubes, 1.5 mL

Conical tubes, 15 and 50 mL

Centrifuges compatible with 1.5 mL, 15 mL and 50 mL tubes

M9, 1X, sterile

Funnel, washed with Sparkleen and 10% bleach before and after using

CellMicroSieve™nylon mesh filter, 35 and 20 µm, washed with Sparkleen and 10% bleach before and after using

Shaker

Calbiochem Protease Inhibitor Cocktail Set I (539131), 100X

PMSF, 100 mM in isopropanol

PBS, 1X

Liquid nitrogen or dry ice and ethanol

For fixed collections, you will also need...

Formaldehyde, 37% (w/v)

Glycine, 2.5 M

Nutator

## Time

7-8 days

## Yield

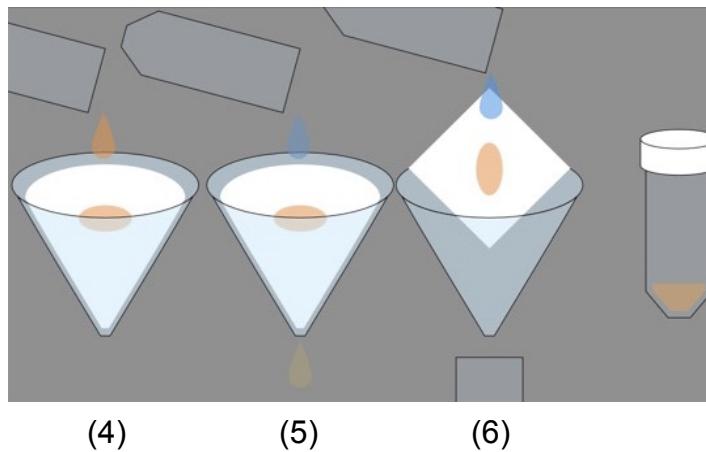
50-150 µl of packed J2-J3 from 10-15 10 cm plates

## Protocol

1. Grow *Pristionchus pacificus* on n<sub>1</sub> 6 cm NGM plates (starter plates) seeded with OP-50. **Tip:** Start with about 6 plates, 5-8 young adult hermaphrodites per plate. Wait about 4-5 days.
2. Transfer 20 young adult hermaphrodites (F<sub>0</sub>) to n<sub>2</sub> 10 cm NGM plates seeded with OP-50. **Tip:** 10-15 plates will give you a good yield.
3. Grow 5-6 days or until most of the F<sub>1</sub> worms reach adulthood. Feed as necessary with **concentrated HB101**. **Tip:** if you notice the worms start to burrow (a crack here or there), wash the worms onto a new plate. **Option:** skip steps 4-9 for a larger yield (100-150 µL). This comes with a higher risk of losing plates to contamination and burrowing.
4. Wash worms off the plates with sterile **1X M9** and onto a 35 µ nylon mesh filter in a funnel over a 50 mL conical tube. The adults will lie on top of the filter while the larvae will flow through and into the tube. **Tip:** be clean and careful, and wash your

filter and funnel well with Sparkleen and bleach first. You will not be bleaching and risk contaminating your worms. **Tip:** let the worms sit in the M9 for a bit before washing off the plate, then swirl to unstick them. If they are still stuck, use a sterile cell spreader to unstick.

5. Rinse the adults with 40 mL **1X M9** over a waste container.
6. Move the filter/funnel to a new 50 mL conical tube. Wash the worms off the filter and into the tube with 50 mL **1X M9** (see schematic and ask someone how to do it).



7. Let the adults settle. **Expected:** at least 200  $\mu$ L of settled adults
8. Discard the supernatant. Wash with **1X M9**, if necessary.
9. Resuspend the settled adults in a 10:1 volume of **1X M9** and pipette them over n<sub>2</sub> 10 cm NGM plates seeded with OP50. **Tip:** swirl the plate before they dry to spread out the adults.
10. Grow for 2 days or until the F<sub>2</sub> are mostly J2 and J3. Feed as necessary with **concentrated HB101**.
11. Add 8 mL of **1X M9** to each plate, and place on a shaker for 5 minutes.
12. Filter the worms through a 20  $\mu$  nylon mesh filter (placed on a funnel) over 15 mL conical tubes (as many as you need). Only the J2-J3 will flow through the filter. **Tip:** Keep the funnel on a 50 mL conical tube when not pipetting to keep it from falling. **Note:** larvae will not settle in a 50 mL conical tube.

13. Leave larvae to settle for at least 15 minutes. Discard supernatant (this is bacteria). Be careful not to disturb the pellet. **Tip:** I will usually leave about 0.5-1 mL of supernatant.
14. Combine your pellets with some **1X M9** into one tube.
15. Wash with **1X M9**. Leave larvae to settle for at least 10-15 minutes. Discard supernatant. Be careful not to disturb the pellet. **Tip:** get as much of the supernatant as possible.
16. Wash with **1X M9**. Pipette 20  $\mu$ L onto a depression slide and check before proceeding. You should not have adults, embryos, or many late stage larvae (L4).
17. Spin at 2000 x g for 1 minute, and discard the supernatant. Repeat until the supernatant is clear.
18. Follow the protocol in  Ercan\_worm\_collection\_vs3 for collecting crosslinked or native larvae (or see Appendix B).

## Recipes

### Bacterial culture

#### OP-50 for seeding

1. Every 3-6 months, streak *E. coli* strain OP-50 from a glycerol stock onto an LB-streptomycin plate and keep at 4° C (tons of protocols online for this. I really like [this one](#)).
2. Make 1 L of LB, split into 4 x 1 L flasks, and autoclave for 45 minutes at 121.5 °C.
3. Take a swab of OP-50 from the plate, and inoculate one flask of LB with streptomycin (final concentration of 25 µg/mL).
4. Leave it at 37 °C or room temperature overnight.
5. Store the bacterial culture at 4° C for a couple of months.

#### Concentrated HB101

See [w Concentrated\\_worm\\_food\\_prep\\_v2.docx](#) from the Ercan lab.

### Worm culture

#### M9, 1X

Add 3 g KH<sub>2</sub>PO<sub>4</sub>, 6 g Na<sub>2</sub>HPO<sub>4</sub> (heptahydrate – 6.8 g), and 5 g NaCl to a bottle or flask. Add milliQ H<sub>2</sub>O to 1 L. Autoclave for 45 minutes at 121.5 °C and let cool. Add 1 mL of MgSO<sub>4</sub> (1 M) before use.

#### NGM plates

1. In a 2 L flask, add 980 ml milliQ water, 3 g NaCl, 20 g agar, (NOTE), and 2.5 g peptone.
2. Add a stir bar inside the flask.
3. Cover the mouth of the flask with aluminum foil, and autoclave for 45 minutes at 121.5 °C.
4. Let the flask cool while stirring on a hot plate set to 55 °C for 30-40 minutes or until it's about bath temperature.
5. While stirring, and next to a flame, add – IN THIS ORDER – 1 ml 1 M CaCl<sub>2</sub>, 1 ml 5 mg/ml cholesterol in ethanol, 1 ml 1 M MgSO<sub>4</sub>, and 25 ml 1 M KPO<sub>4</sub> buffer.
6. Using sterile technique, dispense the NGM solution into 6 cm petri dishes (NOTE).
7. Leave the plates to dry at room temperature for 1 day.
8. Seed with OP-50 (using a 5 mL pipette and Stripettor, 1 drop onto a 6 cm plate, or 4 drops onto a 10 cm plate).
9. Dry overnight by leaving the plates at room temperature.

10. You can store unseeded or seeded plates at 4 °C to make them last longer.

NOTE: To make 10 cm agarose plates for collecting, use 10 g of agar and 7 g of agarose instead of 20 g of agar.

### Other reagents

#### PBS, 1X

I buy this at 10X and dilute with sterile water because I don't trust our pH meter. It's also cheap.

#### Protease inhibitors, 100X

Dissolve Calbiochem protease inhibitor cocktail set I (catalog number 539131) as per the manufacturers instructions to make a 100X solution. Store at -20 °C and add fresh.

Product	Cat. No.	Mol. Wt.	1X Concentration	Target Protease
AEBSF, Hydrochloride	101500	239.5	500 μM	Serine Proteases
Aprotinin, Bovine Lung, Crystalline	616370	6512	150 nM	Serine Proteases and Esterases
E-64 Protease Inhibitor	324890	357.4	1 μM	Cysteine Proteases
EDTA, Disodium	-	372.2	0.5 mM	Metalloproteases
Leupeptin, Hemisulfate	108975	475.6	1 μM	Cysteine Proteases and Trypsin-like Proteases

The above table taken from the manufacturer shows five protease inhibitors in this cocktail.

#### PMSF, 100 mM

Add 348 mg of PMSF and 20 mL of Isopropanol to a conical tube. Nutate to dissolve. Make 1.5 mL aliquots and store at -20 °C. Add to a final concentration of 1 mM as a protease inhibitor. PMSF is inactivated in aqueous solutions. The rate of inactivation increases with increasing pH and is faster at 25°C than at 4°C. The half-life of a 20 mM aqueous solution of PMSF is about 35 min at pH 8.0. Therefore, always add it fresh!

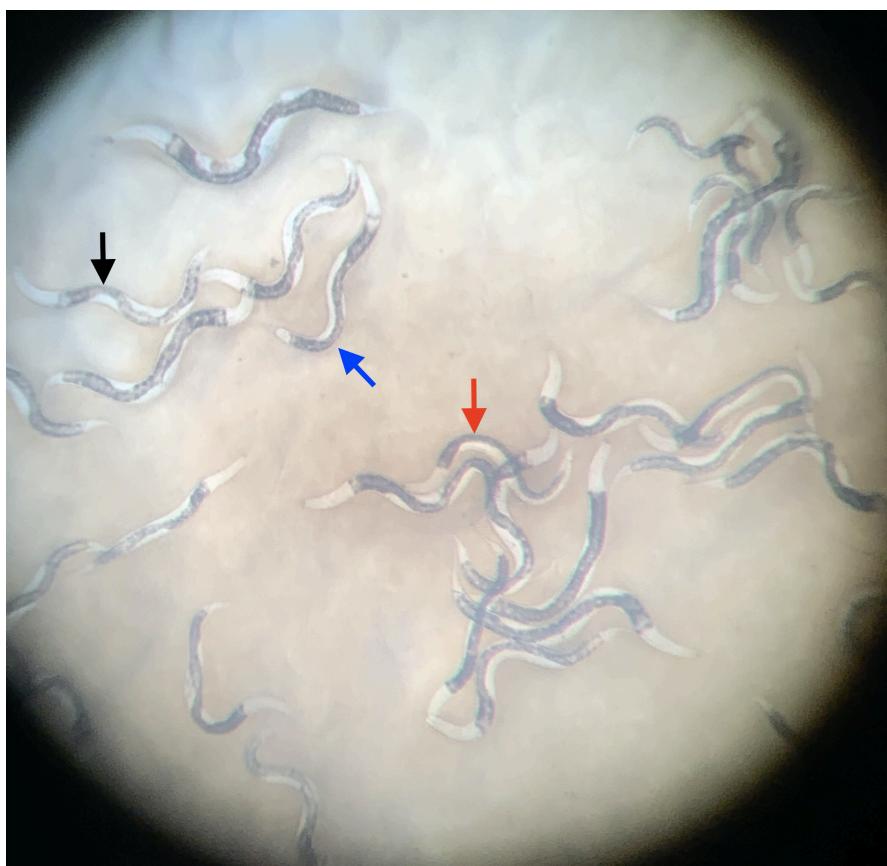
#### Glycine, 2.5 M

Add 93.8 g glycine to 500 mL water, dissolve and autoclave for 45 min at 121.5 °C. Store at room temperature.

## Appendix A. Identifying males and young adult hermaphrodites



Male. Note the single gonad (white) that extends to the tail. Image taken on a dissection scope with a phone camera.



A mix of young adult (YA) to adult and hermaphrodites and males. Red arrow: male. Blue arrow: YA hermaphrodite with a seemingly continuous gonad (white). Black arrow: classic YA hermaphrodite (note the visible didelphic gonad). Images taken on a dissection scope with a phone camera.

## Appendix B. Step 18 (adapted from Ercan\_worm\_collection\_vs3 )

*If collecting uncrosslinked larvae for western, IP, ATAC-seq, etc., then follow these steps*

1. Wash larval pellet with 2-3 mL of **1X PBS**, and spin for 1 minute at 2000 x g.
2. Check that all worms pelleted. Discard supernatant.
3. Transfer about 100  $\mu$ L of larval pellet with 1 mL **1X PBS** to a 1.5 ml tube.
4. Add **protease inhibitors** to 1X and **PMSF** to 1 mM and mix (can add to PBS in step 3 instead as long as it is added fresh because PMSF has a short half life in aqueous solutions). **Note:** You can only freeze/thaw a pellet once. For large pellets, e.g., 200  $\mu$ L or more, split it between multiple tubes to get more samples out of one collection.
5. Spin for 1 minute at 6000 x g. Check that all worms pelleted<sup>#</sup>. Discard the entire supernatant.
6. Snap freeze in **liquid nitrogen** or **dry ice/ethanol**. For liquid nitrogen, dip the tube into a dewar until it stops bubbling. For dry ice ethanol bath, add a layer of drive ice to an ice bucket, and pour enough ethanol into the bucket to submerge the pellet. Dip your tube(s) in ethanol until frozen.
7. Store at -80 °C, and enter information on  Ercan\_Lab\_WormCollections\_Extracts .

*If collecting formaldehyde crosslinked larvae for ChIP-seq, Hi-C, etc., follow these steps*

1. To the larval pellet, add **1X M9** to 9.5 mL.
2. Add **formaldehyde** to 2% final concentration (560  $\mu$ L of 37% formaldehyde to 10 mL).
3. Nutate at room temperature for 30 minutes.
4. Quench formaldehyde by adding **glycine** to 125 mM (550 ul of 2.5 M glycine to 10 mL), and nutate 5 minutes.
5. Spin at 2000 x g for 1 minute. Check the pellet and discard supernatant with a pipette.

6. Wash with 10 mL **1X M9**. Mix well.
7. Spin at 2000 x g for 1 minute. Check the pellet and discard supernatant with a pipette.
8. Wash with 2-3 mL **1X PBS**. Mix well.
9. Spin at 2000 x g for 1 minute. Check the pellet and discard supernatant with a pipette.
10. Transfer pellet to a 1.5 ml tube with 1 mL **1X PBS**.
11. Add **protease inhibitors** to 1X and **PMSF** to 1 mM and mix.
12. Spin for 1 minute at 6000 x g. Check that all larvae are pelleted<sup>#</sup> and discard supernatant.
13. Snap freeze in **liquid nitrogen** or **dry ice/ethanol** (see above).
14. Store at -80 °C and enter information on  Ercan\_Lab\_WormCollections\_Extracts .

<sup>#</sup> If you notice worms on the walls/supernatant of the 1.5 ml microfuge tube, spin for 5 min at 6000 x g. Lower speed for longer is better to prevent hard brake, which shoots up the worms.