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last updated: 10.01.25 (added Appendix A and updated reagents)

Prerequisites
Basics of worm maintenance.

Concepts
Oscheius tipulae lays embryos at the one cell stage, and holds at most a few embryos at a time, leaving us with mostly laid embryos to collect. Plates end up filled with embryos (see Appendix A). To unstick the embryos from the plate, wash with M9 and a cell spreader.

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.

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.

Notes
This protocol is based on development at 22 ˚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

L-shape cell spreaders
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
Erlenmeyer flask
Nutator
NaOH, 5 M
Sodium hypochlorite, 4-6% (Fischer Chemical, SS290-1)
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
10-12 days

Yield
30-100 µL packed early stage larvae

Protocol
1. Grow *O. tipulae* on n_1 6 cm NGM plates (starter plates) seeded with OP-50 until crowded. **Tip:** start with 4 plates, 8-10 L4 larvae each, and place at 22 ˚C.

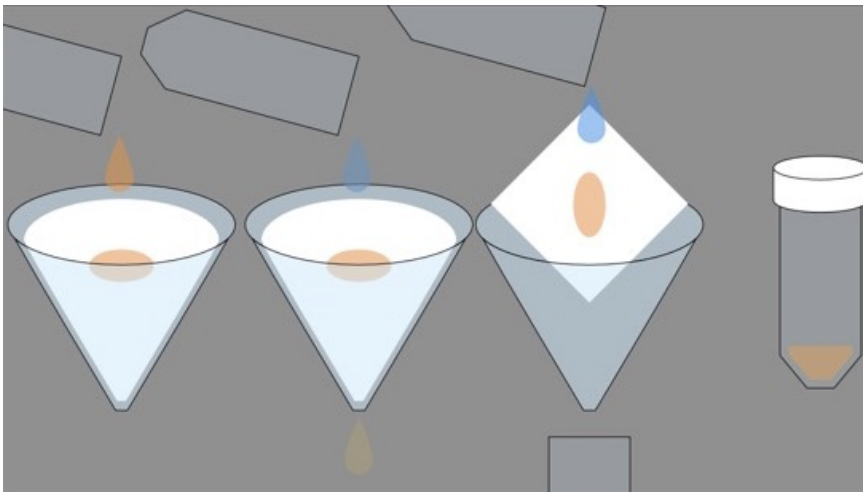
2. Wash off worms with **1X M9** and a cell spreader into a 15 mL conical tube. Spin at 2000 x g for 1 minute. Remove most of the supernatant, resuspend in remaining liquid, and split over $4n_1$ 10 cm NGM plates seeded with OP-50.

3. Grow 4-5 days or until the plates are crowded with embryos. Feed as necessary with **concentrated HB101**. You should see a bunch of embryo piles (see photo album below). **Tip:** if you notice the worms start to burrow (a crack here or there), wash the worms onto a new plate.

4. Wash worms off the plates with **1X M9** and a cell spreader onto a 20 µ nylon mesh filter in a funnel over a 50 mL conical tube. This will catch adults, larger larvae, and embryos while letting smaller larvae flow through.

5. Move the filter/funnel to an Erlenmeyer flask. Slowly wash the filter with 250-500 mL tap water to rinse away bacteria.

6. Move the filter/funnel to a new 50 mL conical tube. Wash worms off the top of the filter and into the tube with 30 mL **1X M9** (see schematic and ask someone how to do it).



(4) (5) (6)

7. Bleach with 0.5 mL **NaOH** and 1 mL **Sodium hypochlorite, 4-6%** per 3.5 mL total volume of washed worms (4.3 mL NaOH and 8.6 mL bleach to 30 mL of washed worms) for 2 minutes on a nutator. **Note:** the adults will not always snap, or disintegrate. The purpose is just to kill them.

8. Spin for 1 minute at 1300 x g, and discard supernatant by pouring.

9. Wash with **1X M9** and spin for 1 minute at 1300 x g. Discard supernatant by pipetting.

10. Wash with **1X M9**, and spin for 1 minute at 2000 x g. Discard supernatant by pipetting. Wash again, if necessary,

11. Resuspend in 30 mL **1X M9**, and split between two 50 mL conical tubes. Nutate for 24 hours.

12. Filter through a 15 µ nylon mesh filter to separate arrested L1s from carcasses.

13. Spin flow through with arrested L1s for 2 minutes at 3000 x g. Discard supernatant by pipetting.

14. Resuspend in a 10:1 volume of **1X M9** and spread it over $4n_1$ 10 cm NGM plates seeded with OP50. **Tip:** swirl the plate before the buffer dries to spread out the larva. **Tip:** remove 5% before filtering, spin down, resuspend in M9, and plate to begin a new collection. Plates should take about 5 days at 22 ˚C to fill up with embryos*.

15. Grow for 24 hours. Feed as necessary with **concentrated HB101**. Check their age by counting their germline cells using DIC microscopy. They should not have started the proliferative stage (probably under 20 cells is good, I'm still figuring out the upper limit. Usually, after 24 hours at 22 ˚C, they have 6-10 germline cells).

16. Proceed to Ercan_worm_collection_vs3 for crosslinking or native collection (or see Appendix B).

*On the 4th day, there will be a considerable amount of embryos, but not as much as on the 5th day. That is, the second wave of embryos will give a bigger collection than the first. A plate that is ready will have a noticeable amount of embryos that have hatched (see photo album below).

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 Concentrated_worm_food_prep_v2.docx.

Worm culture

M9, 1X
Add 3 g KH_2PO_4, 6 g Na_2HPO_4 (heptahydrate – 6.8 g), and 5 g NaCl to a bottle or flask. Add milliQ H_2O to 1 L. Autoclave for 45 minutes at 121.5 ˚C and let cool. Add 1 mL of $MgSO_4$ (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 CaCl2, 1 ml 5 mg/ml cholesterol in ethanol, 1 ml 1 M $MgSO_4$, and 25 ml 1 M KPO_4 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

NaOH, 5 M
100 g NaOH, dissolve in 400 mL H_2O in a glass beaker. Add pellets slowly, the reaction is exothermic and the glass will get hot. Bring the volume to 500 ml, then transfer to a plastic container.

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

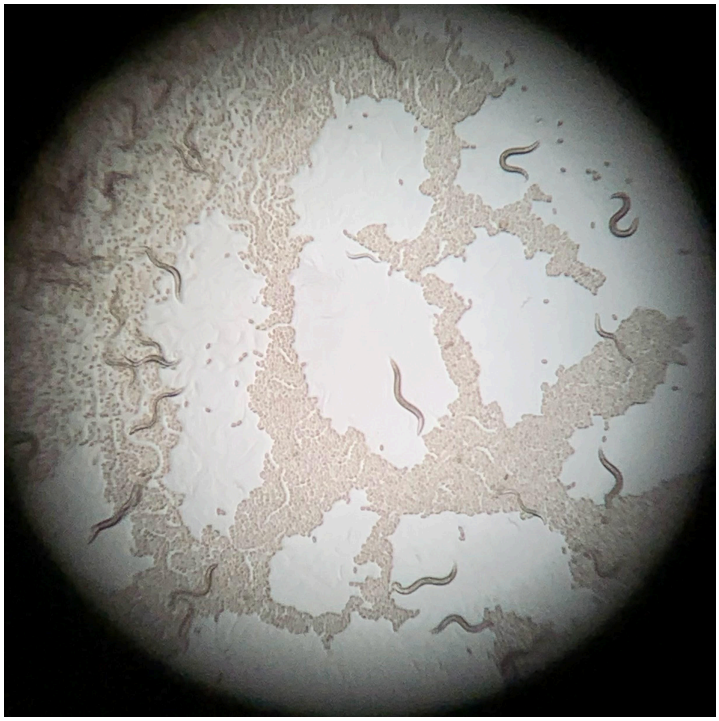
The above table taken from the manufacturer shows five protease inhibitors in this cocktail.

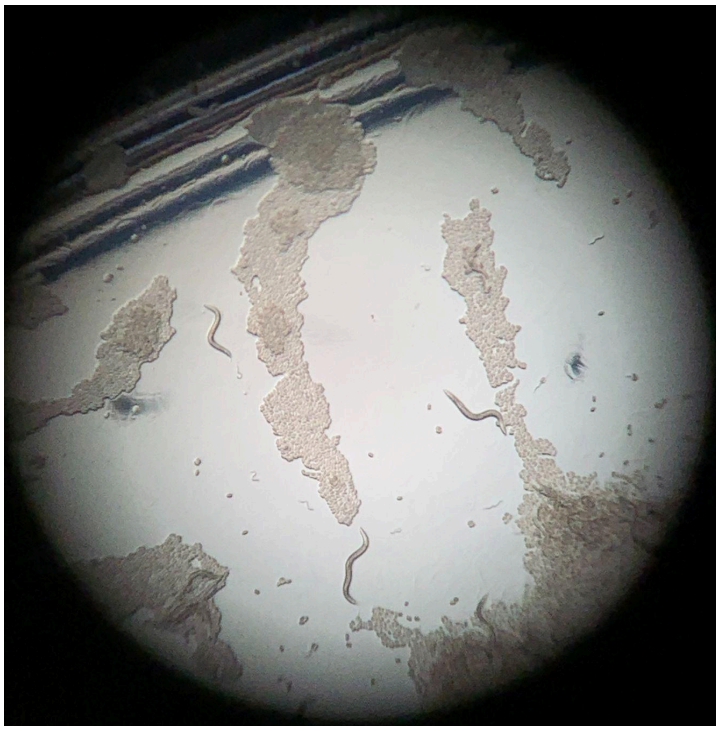
PMSF, 100 mM
Add 348 mg of PMSF and 20 mL of Isopropanol to a conical tube. Nutate to dissolve. Make 1.5 mL aliquots and store at -20 °C. Add to a final concentration of 1 mM as a protease inhibitor. PMSF is inactivated in aqueous solutions. The rate of inactivation increases with increasing pH and is 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. Embryo piles





O. tipulae plates end up with piles of embryos because adults lay embryos at the one cell stage. Embryos stick to plates, and need a cell spreader to get them into solution. Images taken on a dissection microscope with an iPhone.

Appendix B. Step 16 (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 µL 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 µL 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#. 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 µL 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[#] 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.

[#] 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.