

Science Education Collection

***C. elegans* Maintenance**

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Abstract

Caenorhabditis elegans has been, and is still, used to great success as a model organism for studying a variety of developmental, genetic, molecular and even physical phenomena. In order to use *C. elegans* to its full potential, proper care and attention to the basic maintenance of this powerful organism is essential.

In this video you will learn the basic housing and feeding requirements of *C. elegans*, how to correctly handle and manipulate worms using a worm pick and how to freeze and recover important worm stocks. Towards the end of the video we will visit a few applications of modifying the housing, feeding and manipulation of these important animals.

Transcript

Correct care and maintenance of *Caenorhabditis elegans* is essential for successful experiments.

They require very little space, are cheap to house, have high fecundity, and are easy to manipulate. But just because they are simple to keep around does not mean wimpy science. In fact, Sydney Brenner famously called *C. elegans* Nature's gift to science, and since its introduction in 1974 the worm has been featured in a number of Nobel prize winning experiments.

In this video we will demonstrate basic methods for maintaining *C. elegans* in the lab, including: housing and feeding, handling, as well as freezing and thawing of worms.

In the wild, *C. elegans* are found in the soil and feed upon decomposing plant matter. In the lab, it's important to keep nematodes as happy as possible, and so we house and feed them using very specific methods.

C. elegans can be grown at 16, 20, or 25 °C depending upon the requirements of the experiment, and can either be grown on solid or in liquid media. In both cases they are fed OP50, a standardized strain of *E. coli* used specifically for nematode culture in every worm lab around the world.

When grown at 25 °C, *C. elegans* complete their life cycle 2.1 times faster than when grown at 16 °C. A faster life cycle means the worms mature faster, lay more eggs and consume more food. If worms are allowed to starve or become too crowded they enter a larval stage called the dauer stage. Dauer larvae are stress resistant and do not age.

When maintained on solid media, *C. elegans* are grown on agar plates prepared with nematode growth media or NGM media. The day before making plates, inoculate liquid LB media with a single colony of OP50 *E. coli*. Incubate the media at 37 °C overnight with shaking.

To make solid media, measure and combine the appropriate amounts of these ingredients with deionized water in an Erlenmeyer flask.

After autoclaving for at least 15 minutes, let the agar cool to 55 °C in a water bath. Once the media has cooled to 55 °C you should be able to comfortably hold the glass container with bare hands. Using proper aseptic technique, additives like drugs or antibiotics can be added at this time. Swirl to mix. Then, pipette molten NGM into Petri plates until they are 2/3 full. Let the newly made plates dry on the bench overnight.

The next morning pellet the OP50 at 3500 x g for 10 minutes and then resuspend the bacteria in LB media to a 10X concentration. Now, pipette a central lawn onto each plate. Avoid touching the pipet tip to the surface of the NGM and take care not to allow the culture to touch the plate walls. Leave plates to dry on the bench overnight. Once dry, expose plates to UV light to sterilize them. They are now ready to be used when culturing worms.

C. elegans are individually manipulated using a tool known as the worm pick. The pick is typically made from 30 gauge 90% platinum and 10% iridium wire, though some researchers may prefer slightly different metal compositions. To make a pick, start by breaking the tip of a Pasteur pipet to the preferred length.

Cut about 3 to 4 cm of wire and place 0.5 cm of it inside the tip of the pipet. Seal the wire to the glass over a Bunsen burner. The length of the wire protruding from the glass is about 3 to 3.5 cm but can vary according to individual preferences.

Flatten the end of the wire using a hard edge. Then bend the flattened portion upward to form a scoop. Finally, sand the edges of the pick to prevent damaging the worm or the agar.

To pick worms sterilize the wire of the pick on a flame. Then coat the tip with thick, sticky OP50 *E. coli* from an NGM plate. Use care to not puncture or scar the agar surface.

While looking through a dissection scope, lightly scoop the worm onto the flattened, sticky pick until the worm sticks to the pick.

Once the worm is on the pick, immediately transfer by lightly holding the tip to the new surface of a new plate and sliding it across the bacterial lawn. The worm should crawl off the pick. The worm should not stay on the pick for too long or it might dry out.

One of the reasons why *C. elegans* is a popular model for research is because cultures can be stored for long periods of time without any adverse effect.

First, wash freshly starved larvae using 0.5 ml M9 Buffer, gently swirl to loosen all larva and adult animals, and then transfer to a microcentrifuge or cryotube. Then, add equal amount 30% glycerol in M9 Buffer. Finally, pack the vial into an insulated box and store at -80 °C.

To recover worms, remove the tube from a -80 freezer and let thaw at room temperature until the contents fully melt. Pipette the liquid onto a fresh NGM plate with OP50 lawn and incubate at 20 °C. After 2-3 days, transfer 10-15 animals to a new plate and allow them to reproduce for one generation. Collect the progeny and score for correct phenotypes.

Now that we've seen how *C. elegans* is maintained in the lab, let's have a look at how feeding, housing, and handling conditions are modified for experiments.

The Nobel winning discovery of RNA interference allowed researchers to silence any *C. elegans* gene in order to determine its function.

We can induce RNAi in *C. elegans* by first preparing plates with *E. coli* that express target gene dsRNA, which the worms will eat.

Then 4th larval stage worms are transferred to the RNAi plates and allowed to lay eggs. At the desired stage of development the progeny are collected and scored for phenotypes. Since we share about half of our genome with the worm many of the insights gleaned are applicable to human disease.

Because of its quick life-cycle, *C. elegans* is particularly well suited as a model of aging.

First, a time-synchronized population of *C. elegans* is generated, by allowing adult hermaphrodites to lay eggs on NGM plates. The worms lay eggs for 6-8 hr and then are removed.

When worms are at the desired stage they are transferred to new NGM plates containing ampicillin to prevent bacterial contamination and FUDR to prevent reproduction.

From this point forward adult worms are observed every 2-3 days until all worms have died. Dead worms are removed from the plate and the number of live and dead worms are recorded.

Analyzing life span in the context of genetic or environmental insults can yield significant insights into the aging process.

Using lasers it is possible to perform axotomies or the cutting of individual axons, in live *C. elegans* to study how nerve cells regenerate.

But, because worms never keep still, they are placed on 10% agarose pads in solution of microbeads. A cover slip is placed on top. The microbeads increase the coefficient of friction of the pad-coverslip interaction, effectively freezing the worm in place.

The slide is now prepared for performing axotomies. The neurons are brought into view and centered on the microscope. The laser is then fired using the foot pedal. Optimum laser power will sever the neuron without harming adjacent structures. As many axons as possible are cut per animal.

The agarose pad is then carefully removed from the slide, and worms are allowed to recover on a seeded NGM plate at 20 °C. Between 8-48 hr after the axotomy, the neurons can be prepared to score for regeneration. At the distal part of the cut, the neuron forms a stump. However, at the proximal portion the neuron regenerates forming elongated neurites.

You've just watched JoVE's take on basic *Ceanorhabditis elegans* maintenance. In this video we reviewed: housing and feedings of *C. elegans*, handling them, and freezing and recovery of nematodes.

We also took a brief tour through some applications that make *C. elegans* such a powerful research tool. Although *C. elegans* are dissimilar to mammals in many ways, their similar genetic makeup, ease of maintenance, and simple manipulation make them an important model in the quest for understanding mammalian biology and disease. Thanks for watching!