Lewis Lab Protocol book

4/2/2026 | Version 1

This is a living document that has all Lewis lab protocols. As we do new techniques, they will be added to the protocol book. If you are one of the people doing this new fancy protocol, please see the instructions on page one on how to add things to this document. Components of this handbook were taken from the labs of Amanda Gibson, Levi Morran, Richard Morimoto, Erik Anderson, and Michael Koelle.

All things related to the Lewis Lab community are found in the Handbook. This book and the Lewis Lab handbook can be found online via our website.

Lewis Lab CGC Designators

Lab/Strain: LAJ (Lewis Alexander Jordan) | Allele: jlr (Jordan Lewis Research)

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Adding to the Protocol Book

In the Lewis Lab, we use four main model organisms. These are *C. elegans*, *S. marcescens P. aeruginosa*, and various bacteriophages. As such, the lab protocol book is a bit of a beast that encompasses all these protocols. While this book can always be found in the lab, it will also remain on the lab website for your convenience. You can also find these protocol sheets organized individually in the lab OneDrive folder.

If you are using a protocol that is not in the book or is new, please add it to this binder to assist the lab and members who follow you. To be a selfless lab legend and add to the book, do the following steps:

A. Name the protocol appropriately and add notes on what it is for and when you use it.
B. List all needed materials and the estimated time to complete.
C. Use detailed information and write the steps out so someone with our normal lab training can follow them easily. Language does not have to be incredibly formal.
D. At the end of the protocol, make sure to add recipes for any formulas that are needed. In our lab, standalone protocol sheets always include this.

After you finish with the protocol, send it to Dr. Lewis as a Word Document. After this, he will add it to the lab protocol list, integrate it into the long-form lab book (this document), and update the version number. Please do not physically add anything to the lab notebook before sharing it with Dr. Lewis. After he updates the lab book or asks you to do it, he will give you instructions on how to add it to the binder.

Some Potentially Useful Online Resources

From WormBook: The Online Review of *C. elegans* Biology. (Potentially best place to start)
List also partially taken from the Richard I. Morimoto Lab at Northwestern University.

Online Location of the Lewis Lab Manual
jordanlewisphd.com/community-news

This page and the entire Lewis lab protocol manual can be found online at the link above.

WormAtlas

https://www.wormatlas.org/

WormAtlas provides anatomical information of *C. elegans*. The front page lists several useful entry points. One can use the simple text search tool to search the site for information that relates to anatomical terms (e.g. PVT, name of a neuron). Another good way to use this site is to read sections of the “handbook”.

WormBase

https://www.wormbase.org/#012-34-5

WormBase is a major repository for *C. elegans* information, including genomic, genetic, anatomical, people, and literature. Access to information is via a set of Web pages, each of which is specifically designed for a different type of biological knowledge. Further, different information types, when appropriate, are connected horizontally via hyperlinks. One can easily move from one Web page to another. For example, you can start by visiting a genome sequence, click a link to read about a gene that resides in this sequence on a gene page, click another link to review an expression pattern description on an expression pattern page and click again to read about a cell on an anatomy page.
To look up worm strains, genes, alleles, etc. go to: <http://wormbase.org/db/gene/strain>.

WormBook

https://www.ncbi.nlm.nih.gov/books/NBK19662/

WormBook is a comprehensive, open-access collection of original, peer-reviewed chapters covering topics related to the biology of *Caenorhabditis elegans*.
WormBook also includes WormMethods, an up-to-date collection of methods and protocols for *C. elegans* researchers.

Caenorhabditis Genetics Center (CGC)

https://cgc.umn.edu/

Caenorhabditis Genetics Center (CGC) is a resource center for *C. elegans* genetics. It is responsible for gene nomenclature, strain collection and distribution, and genetic map construction. CGC homepage is a portal that has links to these and some other related services useful to *C. elegans* geneticists.

Ordering Strains from the CGC

Strains are sent with a Strain Information Sheet, which gives the genotype, phenotype, culturing conditions and derivation of the stock, as well as a bibliographic reference. Requesters are asked to inform the CGC of the date the strains were received and their condition on arrival. The CGC should be acknowledged in any publication that results from the use of strains acquired from the CGC. The CGC has charges $10 per strain. Although this is not expensive, it does add up over time. If you receive a strain from the CGC make sure you chunk a piece for freezing to add to the lab stock.

Textpresso

https://www.alliancegenome.org/textpresso/wb/tpc

Textpresso allows text searches on primarily C. elegans literature, including published papers, personal communications and meeting reports. Two major features distinguish Textpresso from other literature search tools: that it searches for full-text contents of publications, and in addition to text strings, that it can search for groups of terms (categories).

The simplest way to search using Textpresso is to start with the default settings and type into the query box a text string. For example, if one wants to learn about the regulation of kinases, one can search for "regulate kinase". Textpresso treats the words independently. The default setting automatically appends a wild card to the end of each word thus expanding the search to include any word that begins with "regulate" or "kinase". Further, the default setting is to search for sentences that simultaneously have both groups of words. Textpresso also offers category search and many other advanced features. Users can read the user guide to learn how to use advanced features.

National Center for Biotechnology Information (NCBI) GenBank

https://www.ncbi.nlm.nih.gov/genbank/

NCBI GenBank is a repository of sequences from many phylogenetically diverse organisms including the worm. Search by a simple text string match; follow the appropriate links (Nucleotide or Protein) to download sequences. A GenBank release occurs every two months.

NCBI BLAST (Basic Local Alignment Search Tool)

https://blast.ncbi.nlm.nih.gov/Blast.cgi

NCBI Blast offers a very extensive set of blast services. Here, different types of blast searches can be performed against all available sequences in GenBank database. One can even download programs to install and run local. BLAST finds regions of similarity between biological sequences. The program compares nucleotide or protein sequences to sequence databases and calculates the statistical significance

American Type Culture Collection (ATCC)

https://www.atcc.org/

ATCC is a nonprofit organization which collects, stores, and distributes standard reference microorganisms, cell lines and other materials for research and development. This collection includes bacteria, viruses, fungi, cell lines, and bioproducts.

Bacterial and Viral Bioinformatics Resource Center (BV-BRC)

https://www.bv-brc.org/

The BV-BRC is a comprehensive resource for bacterial and viral infectious disease research. BV-BRC provides integrated data, advanced bioinformatics tools, and workflows to support the scientific community in understanding and combating infectious diseases. Offers advanced bioinformatics workflows, including phylogenetic tree construction, whole-genome SNP analysis, and comparative genomics. Covers bacterial, viral, and associated host/vector data. It includes genomic sequences, annotation, protein structures, clinical study data, and antimicrobial resistance markers.

Literature Search Resources

There are several resources available publicly and through the university that are helpful when conducting literature searches. Please see the services below:

Google Scholar: Best for broad initial searches. Searches across all disciplines.

PubMed: One of the premier free resources for life science searches.

Scopus: A comprehensive database that searches across multiple disciplines.

Web of Science: Great for tuned-in or more refined searches.

Worm Nomenclature 101

Drafted by Cindy Voisine and updated in 2019 by Renee Brielmann
Adapted from the Dr. Richard I. Morimoto Lab at Northwestern University

Outline for C. elegans Nomenclature

1) **Lab Identification**
2) **Gene Names**
3) **Allele/Mutations**
4) **Transgenes**
5) **Multiple Mutations in a Strain**
6) **Proteins/ mRNA**
7) **Phenotypes**
8) **Strains**

1) Lab Identification

The two or three letters in the beginning indicate where the strain was originally isolated or constructed, if it is a modified strain. Some examples include:
CB: Cambridge (The MRC LMB- where Sydney Brenner and co began all this worm stuff)
AM: Richard Morimoto (The lab we used to help find many of our lab protocols)

Each lab also has an allele designator that designates a mutation that they named.
Some common lab allele designations include:
e: Cambridge ("*England*")
rm: Richard Morimoto
The Lewis lab designators are: Strain (XXX) , Allele (YYY)
If you are ever wondering about where a strain came from, please see the CGC Strain List

2) Strain Names

The strain name is next. Following the lab designator, a number is assigned.

- Nonitalicized names consisting of 2 or 3
- Capital letters followed by a number
- Every cross requires a new strain name
- Back crossing does not (specify in database).
- Each independent extrachromosomal array line has a new strain name
- Each independent integrated array has a new strain name

2) Gene Names

Following the strain name, the genotype is designated. This lists all the mutations in the strain and follows several rules.:

- 3 or 4 letters, hyphen, number
- Italicized and lowercase.

- Usually followed by the linkage group or chromosome number
- *C. elegans* has 9 autosomal chromosomes. If you see X listed, it is referring to a mutation that is on the sex chromosomes.

3) Allele/Mutations

Lists the specific alleles and mutations that designate a worm genotype.

- Every mutation has a unique allele assignment
- italicized letter(s) followed by Arabic number
- (gene name, allele location, chromosome location)
- *Mutations that are temperature sensitive can be designated as ts following the allele name
- *Wildtype alleles have + sign in ()
- if deletion takes out more than one gene *Df*
- knockout consortium allele codes are *ok*, *bc*, *tm*

4) Transgenes

- Italicized name with allele *(rm)* prefix, *Ex* and a number
- Integrated arrays are *Is*
- Mossaic arrays are *Si*
- *Can be followed by square brackets with genotypic or molecular information
- *Reporter strains: transcriptional or translational fusions generally not defined; can have p for promoter; gene name followed by two colons and the reporter.

5) CRISPR

- gene name for the location of insertion, followed by () containing an allele name
- *Can be followed by brackets containing insertion information
- *Can be followed by chromosome number.

6) Multiple Mutations in a Strain

- listed sequentially according to linkage
- different linkage groups separated by semicolon
- Heterozygotes: separate mutations on the two homologous chromosomes with a slash; can use + sign for wildtype

7) Proteins/ mRNA

- For Protein, refer to gene name
- non-italicized and capitalized
- those with alternate splicing get letter
- mutant proteins are referred to by the change
- For mRNA, refer to it as the protein's Mrna
- For non-coding RNA, use gene name

8) Phenotypes

You can typically determine the phenotype by name because it will usually correspond to a gene name. For instance, Unc mutants are (uncoordinated), but this can be conferred by several mutations in several *unc* genes.

- non-italicized 3 or 4 letter abbreviation
- which usually corresponds to a gene name
- first letter is capitalized
- when referring to wildtype call it non-
- RNAi phenotypes use gene name with *(RNAi)*

Examples:

JK574, *fog-2(q71) V.*

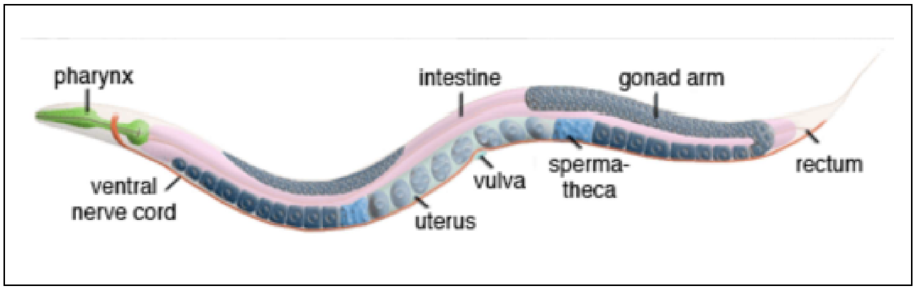
JK designates this as coming from Judith Kimble's lab at the University of Wisconsin at Madison. The strain designator is 574. It has a mutation in the fog-2 gene, at allele q71. Q designates that the Kimble lab also isolated or created the mutant. The fog-2 gene falls on the 5^{th} chromosome.

CF2805, dop-2(vs105) V; dop-4(ok1321) dop-1(vs100) dop-3(vs106) X.

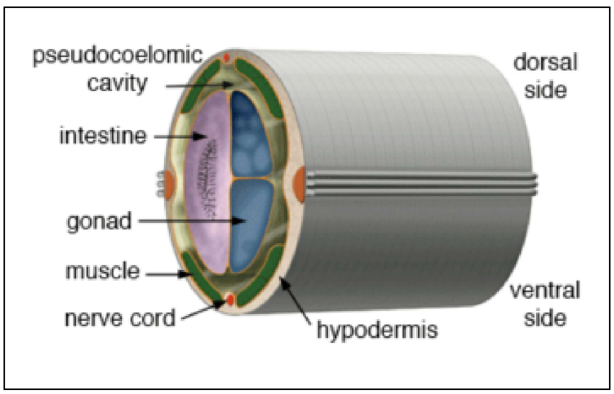
CF designates that this came from Cynthia J Kenyon at Calico Life Sciences in San Francisco, California. The strain designator is 2805. It has mutations in the dop-2 gene at allele vs105, the dop-4 gene at allele ok1321, and the dop-3 gene at allele vs106. All mutations designated vs were first discovered in the lab of Michael Koelle at Yale, and ok designates discovery first in the lab of Robert Barstead at the Oklahoma Medical Research Foundation. The first mutation is on the 5^{th} chromosome, while the other 3 are on the sex chromosome (X).

C. elegans **Basic Anatomy**

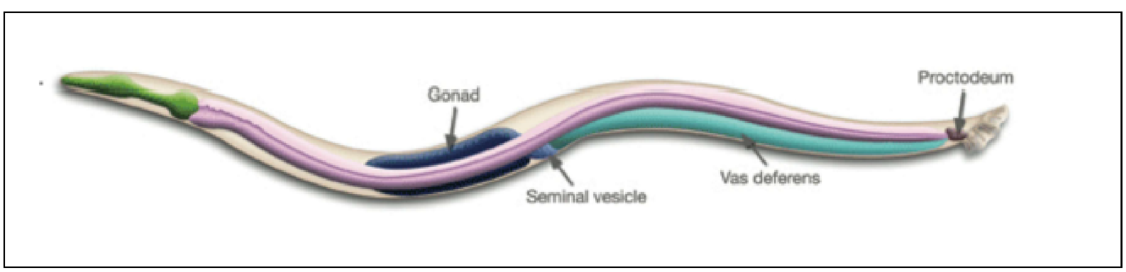
Artwork by Altun and Hall, www.Wormatlas.org



Anatomy of an adult hermaphrodite *C. elegans*



Cross-section through an adult hermaphrodite *C. elegans*



Anatomy of an adult male *C. elegans*

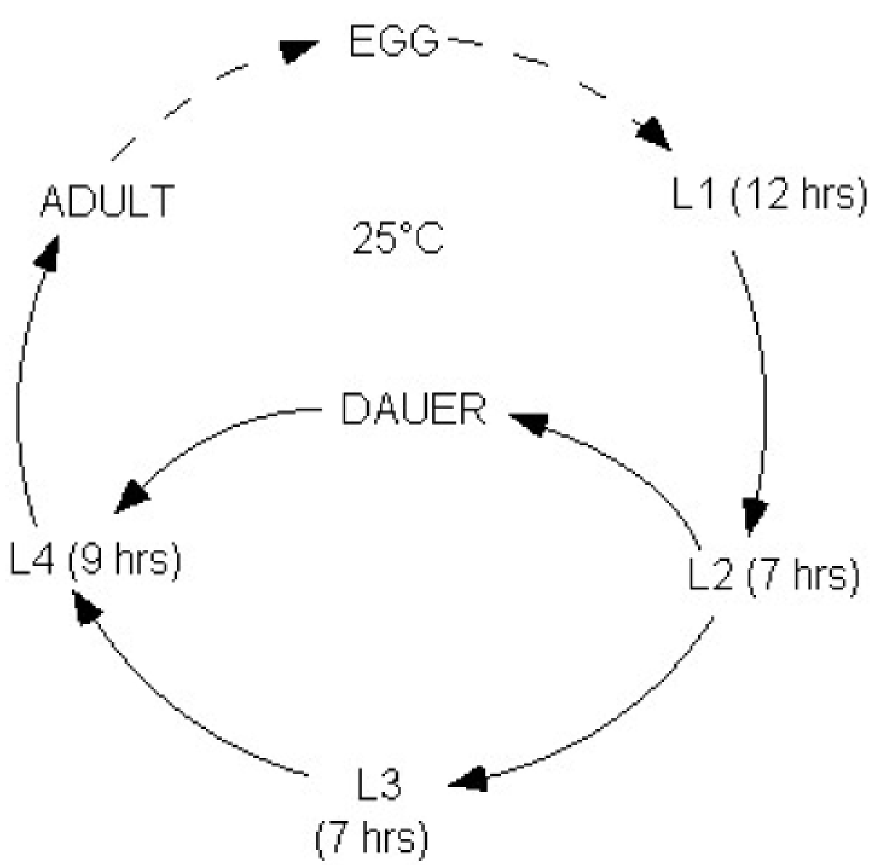
***C. elegans* Lifecycle**

Table I
Growth Parameters of the *Caenorhabditis elegans* Life Cycle[a]

Temperature (°C)	Embryogenesis (h)	Molts (h posthatch)				First eggs laid (h posthatch)
		L1–L2	L2–L3	L3–L4	L4–adult	
16	29[b]	24	39	54.5	74.5	94–97
20	18[b]	15	24	34	46	59–60
25	14	11.5	18.5	26	35.5	45–46

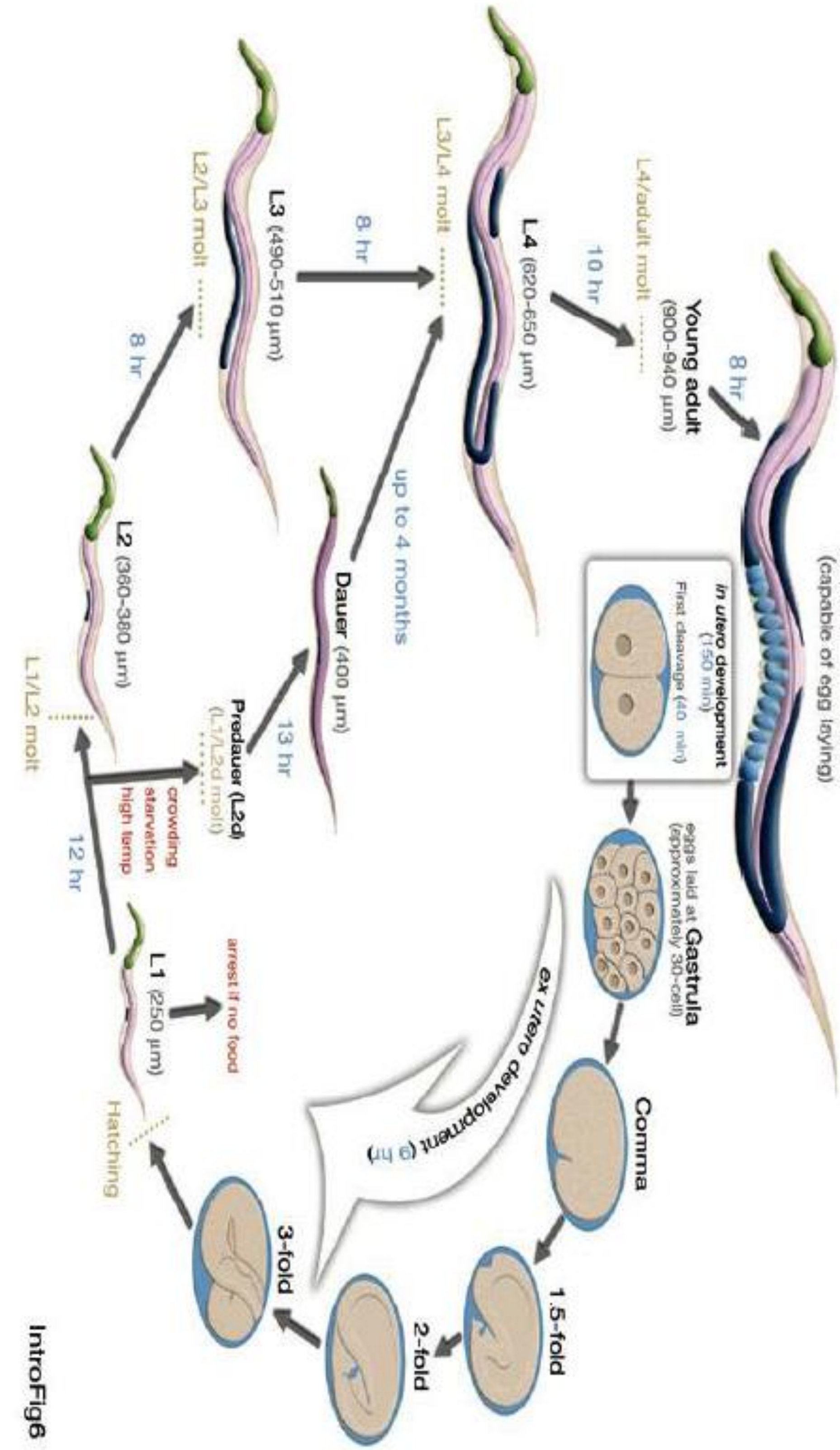
[a] Based on Hirsh *et al.* (1976, Fig. 2).
[b] Calculated by multiplying 25°C embryongenesis time by 20 and 16°C growth rate factors of 1.3 and 2.1, respectively.

Scanned from the book: Methods in Cell Biology, volume 48: *Caenorhabditis elegans*: Modern Biological Analysis of an Organism, Academic Press, 1995.



Basic life cycle of *C. elegans* at 25°C (From Riddle, D. L. (1988). The Dauer Larva. *In* The Nematode *Caenorhabditis elegans* (W. B. Wood, ed.), pp. 393-412. Cold Spring Harbor Laboratory, Cold Spring Harbor, New York, 1988.

Life cycle of *C. elegans* at 22 °C (From http://www.wormatlas.org/handbook/fig.s/IntroFIG6.jpg).



Identifying C. elegans Larval Stages

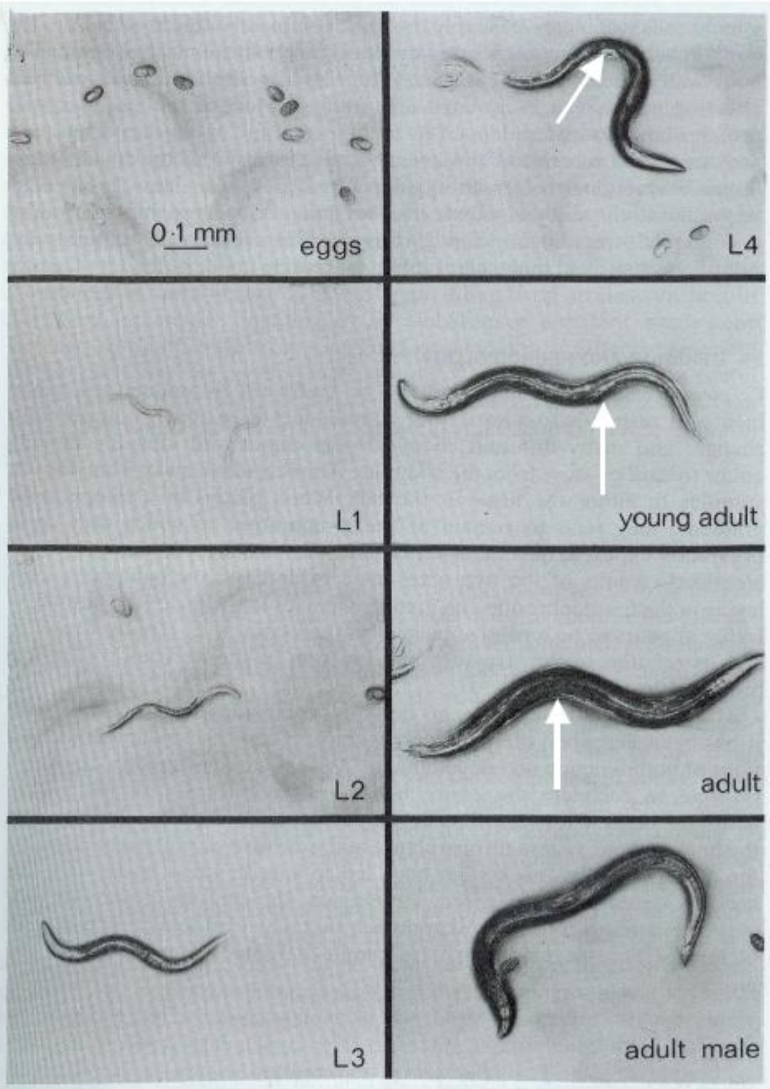
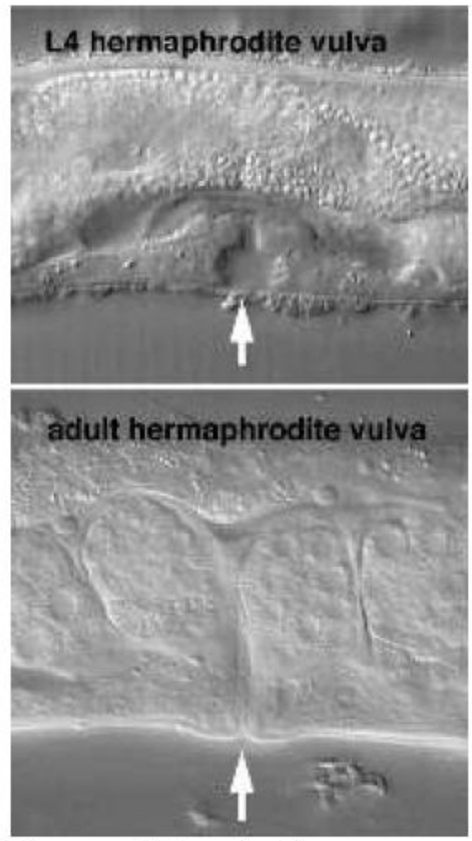


Figure 7 Eggs, larvae, and adults of *C. elegans*. All panels except lower right show hermaphrodites. Bright-field photomicrographs. (Courtesy of J. Sulston.)

Scanned from <u>The Nematode *Caenorhabditis elegans*</u> (W. B. Wood, ed.), pp. 393-412. Cold Spring Harbor Laboratory, Cold Spring Harbor, New York, 1988.



L4 versus adult hermaphrodites, arrow indicates the vulva.

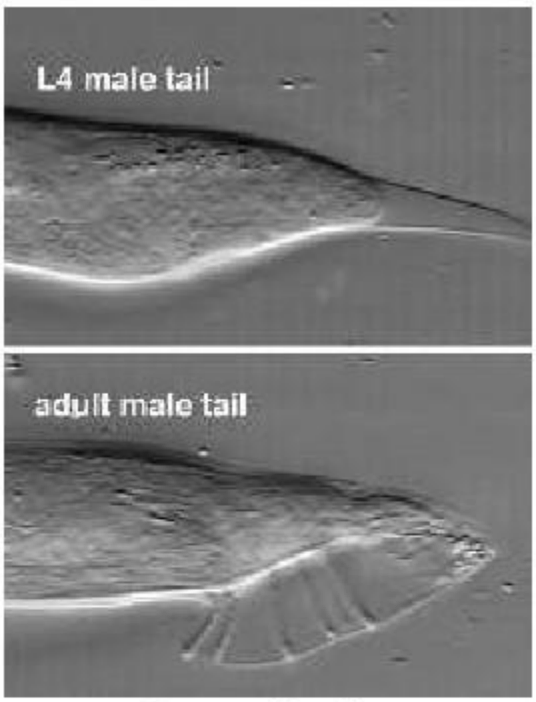
From Cold Spring Harbor Laboratories *C. elegans* Course 2007

L4 stage larvae can be distinguished from adults because the larval cuticle covers the developing adult sexual organs. In the L4 hermaphrodite, the developing vulva appears as a clear indentation (the '**white crescent**') in the middle of the body (Fig. 5), in the adult the vulva appears as a **protrusion in the middle of the body** on the ventral side (From Cold Spring Harbor Laboratories *C. elegans* Course 2007).

Hermaphrodites vs. Males

Hermaphrodites can be identified based on the presence of eggs in the uterus, a vulva on the ventral side roughly in the middle of the body, and a long whip on the tail. Males can be identified because their tail is fan-shaped, they lack a vulva, and they are slightly smaller than hermaphrodites. In addition, in the male the gonad displaces the dark gut on the ventral side and creates a white stripe from the midbody to the tail. (From Cold Spring Harbor Laboratories C. elegans Course 2007)

NOTE: Males are quite rare in most lab strains (only about 0.02% of wild-type populations) so you will have trouble finding them. Sometimes you may see more males in a strain during stressful conditions such as starvation or heat shock. See the “Generation of Males” for protocol for methods to generating males.



L4 versus adult males.

In the L4 male, the tail is enlarged, the fan appears to be contained in a cellophane bag (the L4 cuticle) and a whip is present (Fig. 6), whereas in the adult male, the fan is unfolded and there is no tail whip (From Cold Spring Harbor Laboratories C. elegans Course 2007).

From Cold Spring Harbor Laboratories C. elegans Course 2007

<u>Maintaining and Storing *C. elegans*</u>

From Cold Spring Harbor Laboratories *C. elegans* Course 2007
Adopted from the Morimoto lab at Northwestern University

Maintain strains by picking ~10 worms from a clean plate to the unseeded portion of a fresh plate. Worms will crawl onto the fresh food. Chunking is also an easier option, although it should not be used to maintain extrachromosomal or heterozygous strains.

Short-Term (bench top or 20°C incubators)
Worms kept at 20°C will eventually starve the plate. Such a plate can be maintained for a week or two in the incubator, but will eventually dry out, and the worms will die. Generally, you always want to use an incubator if available. You will know the plate has exhausted its food supply when you stop seeing worm tracks in the lawn. This will arrest worm development except for L1s, which will go into Dauer from stress conditions. For notmal fecundity worms, you will move them to a new plate every 3 days.

Long-Term (15°C incubator)
Freshly *starved*, clean plates can be sealed with Parafilm and stored at 15° for many months, up to one year if you are lucky. Plates must be fully starved; otherwise bacterial growth on an unstarved plate will use up the oxygen in a sealed plate and kill the strain. Bacterial contamination often causes strains to be sick or arrest the growth of worms in the short term, but they can often be recovered. Fungal contamination will usually kill a strain stored at 15°. Therefore, only store clean plates and check 15° stocks once a month for fungal contamination. When doing experimental evolution, always keep the plates from the last passage at 15°C in case something goes wrong.

Cryogenic Storage (-80°C Freezer)
Any strain you value should be maintained in a permanent frozen collection. In the Lewis lab, common stock frozen strains are stored in the –80 °C stock freezer in the master bacteria box. For how to cryogenically preserve nematodes, see the freezing protocol later in this manual.

Dried Chips
Although dried agar chips are not a recommended form of storage, everyone resorts to it unintentionally. At some point, you will realize you needed a strain from a month ago, and you find the box with the correct plates, but they are all dried ☹. You can occasionally recover such strains by rehydrating the chip. If the agar chip is completely dried, it rarely yields viable worms, but if there is any part still adherent to the plate you can still recover the worms. Simply add DI water and let soak for a few hours. Pour the excess water onto a seeded agar and let it soak in. Cut up the rehydrated chip and flip the chunks onto fresh seeded plates. In a couple of days a few worms might crawl out. But it may be a good idea to look into replacing them as well.

NOTE: Any strains kept in the 15ºC or 20ºC incubators should be clearly labeled. Boxes and plates should be checked at least once a week for contamination. Worms should NEVER be placed at 4°C or 37°C (unless doing heat shock at 37°C).

Contamination in the Lab

Modified from Cold Spring Harbor Laboratories *C. elegans* Course 2007
and adopted from the Morimoto lab at Northwestern University

Without a doubt, you will have some form of contamination at some point in your experiments. Although most contaminants do not harm the worms, it can make viewing the worms on the plate quite difficult. Given that we are a species interactions lab, this can also ruin some experiments.

Fungi
Mold and other fungal contamination can generally be chunked out of plates.
However, if there are spores present, the best way to clean a strain is to serially transfer worms to a new plate every 30 minutes for a couple of hours. This way the worms will clear any contamination in their guts and hopefully move away from any carried with your pick.

Non-OP50 Bacteria and Yeast
It is very difficult to cure strains of slime by serial transfer but it can be done, particularly if you are in the middle of a cross and cannot afford to kill a lot of worms by bleach treatment (Bleach or Bleach:NaOH). However, it is usually more effective to decontaminate worms in alkaline hypochlorite solution. This can be done with either an entire contaminated plate or several gravid hermaphrodites. See protocols later in this manual. Some strains are so slow-growing or slow-moving that the alkaline hypochlorite method will not work (L1s will never crawl onto the seeded portion of the plate). In this case, you have to try the serial transfer method.

Dust Mites
Mites eat nematodes, import fungus, and could cross-contaminate worm strains by carrying worms between plates. In some labs, every couple of months
there is generally a couple mite sightings. Thankfully, I have only experienced one in my career. Typically, these are isolated occurrences. However, there can be bad outbreaks that contaminate entire incubators. If this happens *everyone* must check all of their plates, *pick* worms from any infested plates onto new plates (chunking could transfer mites or eggs onto the new plate), and clean out their boxes with ethanol and/or
bleach. Any contaminated plates are sealed with Parafilm and disposed of in biohazard bags. Also, wear gloves or wash your hands after handling it. Mite paper is also an option, and can be ordered if, for some reason, issues persist or are recurrent over a short time period. Generally, mites are most likely to come from a shipment of contaminated plates, so please check all incoming shipments before opening.

Creating a Worm Pick

From Cold Spring Harbor Laboratories *C. elegans* Course 2007

Cut a 1-inch segment from the spool of platinum wire (thick or thin). Insert a little less than a quarter of the wire fragment into a short-nosed glass pipette. Hold the pipette tip over the flame of a burner and melt the glass around the wire (Fig. 1). Hold the wire horizontal with pliers or tweezers.

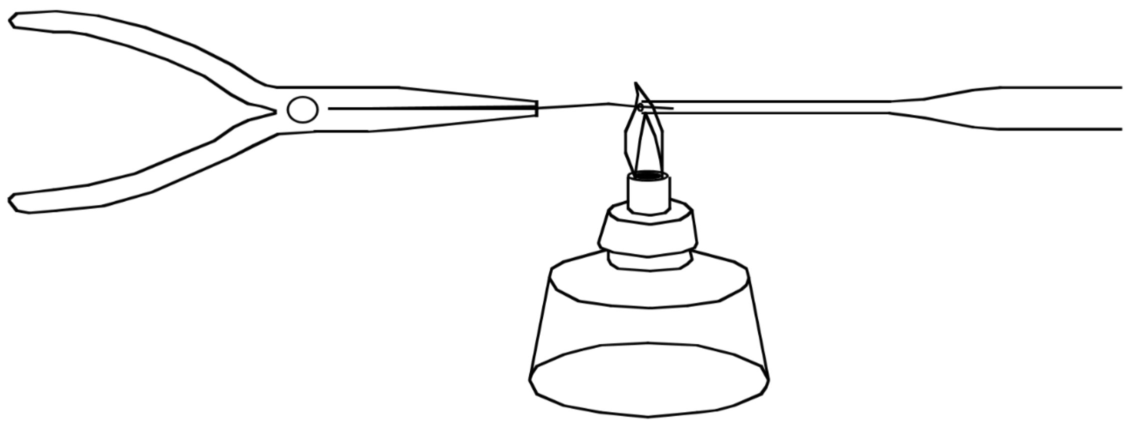


Figure 1. Attaching the platinum wire to a glass pipette.

Next, flatten the tip of the wire into a disc. Grab about a millimeter of the tip of the wire with a pair of jeweler's pliers. Squeeze with all your might. If you haven't much might, place the nose of the pliers on the bench and tap the tip of the pliers with a heavy metal object like the handle of a pair of scissors. This will flatten the wire into a spoon shape at the end (Fig. 2). Bend the pick so that it is angled as shown in Figure 2.

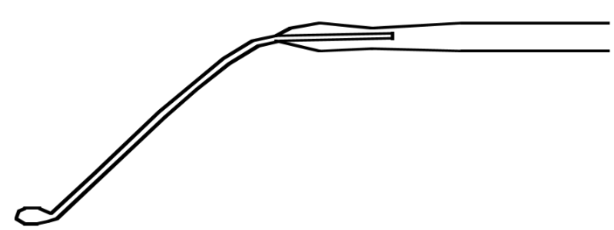


Figure 2. Shaping your pick.

Make sure you label your pick! Picks are like wizard wands; each is unique to the wielder! Please don't use anyone else's pick without their expressed permission!!

Picking Worm

From Cold Spring Harbor Laboratories *C. elegans* Course 2007

C. elegans eat bacteria. In the laboratory we use a crippled strain of *E. coli*, called OP50, that has a uracil auxotrophy, the auxotrophy causes the bacterial lawn to be thinner and stickier than wild type *E. coli*. The reduced bacterial growth allows one to see the worms on the surface of the plate easily and the bacteria are sticky enough to pick the worms up on the pick. The only manual skill one needs to perform *C. elegans* genetics is to move worms from one plate to another.

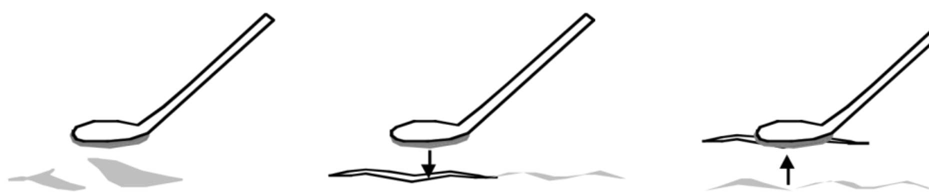
Place your plate of worms under the microscope.

Sterilize your pick in a flame (alcohol burner). Let it cool a couple of seconds.

Remove the lid of the plate.

Use the flattened tip of your worm pick to pick up a worm. Worms are not scooped up on the top surface of the flattened tip of the wire but are rather adhered to the bottom surface of the pick by a layer of sticky bacteria (Figure 3).

Figure 3. Moving worms



Pick up a wad of bacteria on the bottom of the pick by touching the pick to the bacterial surface.

Touch the bottom of the pick gently to the worm.

The worm will stick to the bacteria on the underside of the pick

Close the lid of your plate and fetch a fresh plate from the stack. Open the lid and focus the microscope on the surface of the plate. Place the worm onto the surface of the plate by touching the worm gently to the agar. If the worm doesn't adhere to the plate, a gentle wiping motion can usually dislodge the worm. Try NOT to break the surface of the agar. If the worms find a break in the agar they will burrow into the agar. Soon all of the worms will be inside the agar and you will not be able to pick worms off the plate.

NOTE: When cloning (or singling) worms, be sure to carefully check that an L1 or egg was not carried with the individual, because then the resultant population arises from two non-identical individuals. This is probably the most common mistake of a beginning worm geneticist.

NOTE: Label the plates on the base or side but not the lid since the lid may get separated from the plate.

If you need to create a pick, please see the “Creating a Worm Pick” Protocol.

Nematode Chunking Protocol

Notes: Used to transfer nematodes to a new food plate quickly.
*** For precise worm movement, use the pick protocol.

Time: Depends on the number to be chunked, usually ~10-30 min.

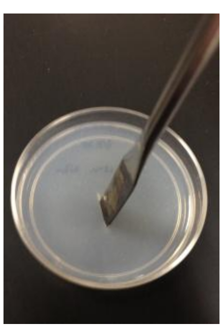
Materials:
Bunson Burner
Ethanol Jar
Marker
Plate (Whatever size you need)
Stainless steel spatula

Procedures:

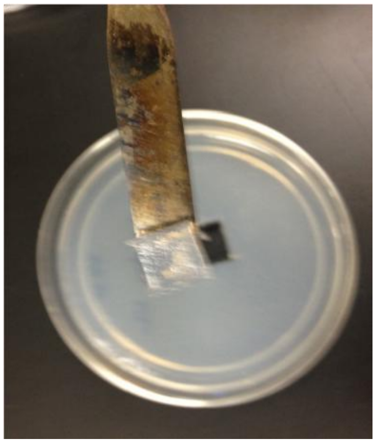
1. Before starting, make sure the bottom of the plate to which you are moving the worms is labeled correctly. -> See lab labeling protocol.
2. Have both your old plate (starved worms, no bacteria) and new plate (no worms, bacteria) ready. Make sure to check for a lawn on the new plate.

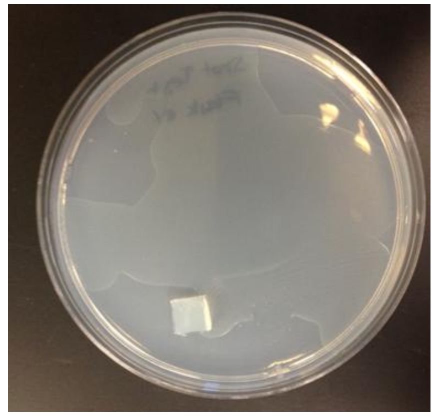
The new plate on the left has a full visible lawn. The older plate on the right has exhausted its lawn.

3. Make sure that you have the tools needed for this protocol ready. Lite the Bunsen burner before opening any plates. Ethanol jar should be relatively close to the flame, but not dangerously.
4. Place the spatula in the jar of ethanol and then pass the spatula through the flame to sterilize it.
5. After the flame has extinguished from the spatula, use it to cut through the agar in the center of the plate and cut out a ~1 cm2 area. Ideally, this area will be a square, but you can also take a piece from the curved edge of the plate.



6. Remove the cut area using the spatula and place the agar from the old worm plate at the edge of the bacterial lawn of the new plate WORM SIDE DOWN.





7. After you place the agar chunk down, hold the spatula in the flame of the Bunsen burner to melt/burn any remaining agar and worms.
8. Place the spatula back in the ethanol to sterilize again before preparing the next sample.
9. You can also use these chunks for removing fungal contamination quickly. See the “Chunk and Go” protocol for that process.

Note: If you want to take one plate of nematodes and make multiple plates, multiple chunks can be removed from the same plate.

Nematode Washing Protocol

Notes: This is the protocol for moving worms via liquid media. This protocol is included in the concentration count, bleaching, passaging, and mortality protocols. To move worms via solid media, use the chunk protocol.

Time: 30 min to 1 hr

Materials:
Centrifuge Tube (15mL)
Cut micropipette tips (wide bore or cut)
M9
Micropipette

Procedures:

1. Using a cut 1000ul tip, add 3ml M9 to wash the synchronized plates
2. Tilt the plate and remove the M9, putting it into centrifuge tube
3. Use a new tip between all worm populations
4. Once worm populations are washed into a tube, centrifuge @1000rpm for 1min
5. Remove the tubes from the centrifuge
6. Using a new, uncut tip for each tube, remove the supernatant
7. You will now be left with a concentrated worm pellet. You can plate the pellet to take all worms, or you can do concentration counts to move a certain amount. You can also split the pellet between multiple plates to get multiple populations.

Nematode Freezing Protocol

Notes: Used to prepare nematodes for long-term -80°C storage.

***For this to be successful, your worm population needs to be mostly starved L1s and L2s. You want to see many young worms, some eggs (maybe), some adults, and a little remaining bacterial lawn. At 20°C, it takes around 24 hours for egg to L2 transformation. Typically, 35-45% of frozen worms will be successfully recovered.

Time: 1 hr.

Materials:
M9
Cryovials
Cryopen
Freezing Solution
P-1000 Micropipette
1000 µL tips
15 mL tubes
Ice
Styrofoam box
Cardboard or plastic freezer box

Procedures:

1. Before starting to wash, fill an appropriate container with ice.
2. Wash your plate with 3mL M9. -> Refer to Worm Washing Protocol
3. Pour the liquid off or use a micropipette tip to suck it up off the plate into a 15mL tube. You want to remove as many individuals as possible.
4. Put the tube on ice for 30 minutes.
   (This is a good time to label your cryovials with the cryoware pen)
5. After 30 minutes, add the freezing solution. You want to add 1.5 times the volume of M9 in your tube. -> Refer to freezing solution protocol
   (For instance, if 3mL of worm M9 solution, add 4.5mL of freezing solution)
6. Cap the tube and shake well to mix
7. Use a cut tip to put ~1mL of the mixture into each cryovial.
8. Put the filled cryo tubes in a Styrofoam box.
   (You can put them directly in the box as you fill them)
9. Shake the box to mix the contents of the vials before moving to the -80
10. After 3 days, move the vials to the regular cardboard freezer box.

You should always check your freezes within 2 weeks of completing the freeze to check viability.
-> Refer to the Worm Thawing Protocol.

Nematode Thawing Protocol

Notes: Used to reanimate nematodes that have been in -80°C storage.
***Typically, 35-45% of frozen worms will be successfully recovered.

Time: 30 min

Materials:
OP50-seeded NGM plate (1 per tube to be thawed)
Marker (to label plates)

Procedures:

1. Remove frozen vials from -80°C freezer and place them on the lab bench. (Thaw one vial per strain/population/treatment you need)
2. Allow the vial to thaw at room temperature until all ice has turned liquid.
3. After thawing, pour the contents of the vial onto one large NGM plate that already has an OP50 lawn. You should see worms wiggling after just a few minutes. (To help the liquid freezing solution dry on the plate, leave the plate cracked on the edge closest to the thaw puddle on the bench top.)
4. After 2-3 days, transfer 10 nematodes individually to separate plates. Allow the nematodes to reproduce for one generation and score the progeny to gauge recovery. Some mutant strains do not survive freezing as well as others.

Bacteria Freezing Protocol

Notes: Used to prepare bacterial cultures for long-term -80°C storage.
***Some bacteria require different protocols than these standard ones. ***

Time: 30 min.

Materials:
Cardboard or plastic freezer box
Cryovials
Cryopen
50% Glycerol Solution*
P-1000 Micropipette
1000µL tips
Vortex Mixer

Procedures:

10. Prior to starting this procedure, set up and label your cryovials on the benchtop. This helps you stay organized during the protocol. ->See lab labeling protocols
(Make sure you use the Cryopen and not a regular marker)
11. Use the micropipette to take 500µL of your preprepared glycerol solution and add it to the sterile cryovials you have prepared.
(By doing this step first, you can add glycerol to each tube with one pipette tip, as opposed to changing the tip between each tube)
12. Use the micropipette to take 500µL of your overnight bacterial culture and add it to the sterile cryovial you prepared. -> Culturing Bacteria Protocol
13. Close the tube and vortex vigorously to ensure that the culture media and the glycerol solution have mixed thoroughly.
14. Put the filled cryo tubes in a freezer box and move them to the -80 °C.
(You can put them directly in the box as you fill them)

Solutions used in this Recipe*:

50% Glycerol Solution

1. Measure 250mL of pure glycerol and add it to the bottle
2. Measure 250mL of DI water and add it to the bottle
3. Cap and shake vigorously until well mixed. Be sure the outside of the bottle is DRY before shaking. Wet bottles can slip from hands easily.
4. Add autoclave tape and autoclave for 20-30 minutes on liquid cycle
(Don't screw cap all the way)
5. Properly label the bottle and place in storage

<u>Growing Bacterial Cultures with LB</u>

This is the general protocol for growing bacterial cultures in the lab. We use LB to grow *E. coli*, *S. marcescens*, and *P. aeruginosa* in culture.

Time: 30 min prep, 1 day until completion

Materials:
Inoculating Loop
LB Broth
Serological Pipette and controller

Procedures

1. We usually start bacterial cultures from a frozen tube in the -80°C, a freeze-dried inoculating loop (like a kwick stick), or a plate that has been shipped to us.
    - If you go from a quick stick, then go to step 2.
    - If you go from a frozen tube, advance to step 3
    - If bacteria are already on a plate, go to step 4.
2. Following the instructions on the kwick stick (or other dry freeze product), prepare the loop and inoculate a streak plate.
    - Place the plate in the incubator overnight at 37°C.
3. If you do not have a streak plate made, go to the -80°C freezer and locate a freezer tube with the species you need.
    - Make sure you have on Cyro gloves and then remove the tube.
    - Quickly, take a sterile loop and dip it into the freezer tube.
      (Make sure that the tube does not thaw completely)
    - Once there is media in the loop, streak that media onto the unseeded petri dish with LB agar. In a pinch, you can also use NGM plates.
    - Put the plate in the incubator overnight at 37°C.
      Note: (By creating a streak plate, we are able to pick a single colony of bacteria to start our liquid culture. If we went directly from the freezer tube, we would not be able to control for how many colonies start our culture or for contamination by strains outside of the one we froze.)
4. Use a serologic pipette to place 200mL of LB into a flask.
5. Then, use a loop to pick one colony from the streak plate and inoculate at 37°C in the shaking incubator overnight.
   (The streak plate can be kept at 4°C (Cold Room) in a labeled box with a lid for several weeks before it needs to be replaced.)
6. Make sure you also place some LB (~25mL) in a tube in the incubator with the culture flask as a control to check for contamination in the LB.
7. You can use the resulting bacterial culture to seed plates for a few weeks by keeping it sealed tightly with Paraffin film or aluminum foil.

Seeding Nematode Growth Media (NGM) Plates

This is the general protocol for seeding NGM plates with *E. coli*. If you are trying to do an assay with a pathogen, please see the appropriate protocol for the bacteria in question.

Time: 30 min prep, 1 day until completion
Materials:
Bunsen Burner
Fume Hood
L-Spreader (metal or sterile)

Procedures

1. Before seeding bacterial lawns, you must inoculate a culture of LB with OP50 or 0P50-1, depending on what you are doing. -> Refer to "Culturing bacteria in LB".
2. You can place plates in stacks to seed them. For 10cm plates, a stack of 10 is best. For 6cm plates, this should also work. However, for mini plates, you may have difficulty stacking them in this way.
    - Plates may have condensation on the lid from temperature changes. If so, you can flip the plates over, tap them on the bench to ensure the condensation is only on the lid, then remove the lid and tap the water off.
    - Alternatively, you can place the plates in the fume hood with the tops off for ~20-30 minutes to allow them to dry off.
3. Using sterile technique, pipette the desired amount of OP50 onto the dried NGM plates. The amount will depend on the size of the plate. Generally, for 10cm plates we use ~200 µL of OP50 per plate, for 6cm plates we use ~100 µL, and for 3.5cm plates we use ~50 µL.
    - One helpful tip is to take a stack of 5 10cm plates and suck up 1mL of culture in a P-1000 micropipette. Then dispense roughly 200µL in each plate in the stack from bottom to top.
4. Use the L-spreader to spread the culture media around in a circle in the center of the plate. Try to keep the OP50 in the middle of the plate like in the photo below but more symmetrical on each side.
   (if the OP50 touches the side of the plate, there is greater risk of contamination, and worms can leave the lawn to crawl onto the sides of the plate)
5. Plates can be incubated in one of three ways:
    - Overnight or as little as 8-12 hours at 37°C.
      Overnight in the incubator at 28°C (More than 2 days will dry them out)
    - 24-48 hours at room temperature
6. Seeded plates should be clearly labeled in a box or grouped with tape and can be kept in the 4°C for several weeks before drying becomes an issue.

Chunk and Go Contamination Protocol

Notes: Used this protocol to remove mold contamination without having to use the full bleach or bleach and NaOH protocol.

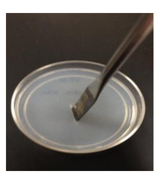
Time: ~1-2 hrs

Materials:
Bleach Solution*
Bunson Burner
Ethanol Jar
Marker
Parrafin Film
Petri Dishes
Stainless steel spatula

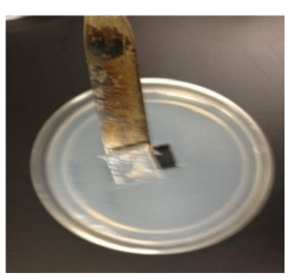
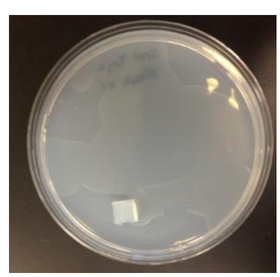

The plate on the left shows typical fungal contamination vs. bacterial (right). Fungal contamination usually appears more "fuzzy" and grows patchier and slower.

Procedures:

15. Before starting, make sure the bottom of the plate to which you are moving the worms is labeled correctly. -> See lab labeling protocol.
16. Have both your old, contaminated plate and your new plate. Make sure there is a visible lawn on the new plate, and you have all the tools from the chunk protocol.
17. Place the spatula in the jar of ethanol and then pass the spatula through the flame to sterilize it.
18. After the flame has extinguished from the spatula, use it to cut through the agar in the center of the plate and cut out a ~1 cm^2 area. Ideally, this area will be a square, but you can also take a piece from the curved edge of the plate.
    (Looking under the scope before the chunk can help you find a suitable area.)
    a. If the original contaminated plate is older than a month, do a larger chunk (5 cm^2) to increase the likelihood of transferring live worms. It might take longer (up to 24 hours) for them to crawl from the chunk to the new plate.




19. Remove the cut area using the spatula and place the agar from the old worm plate at the edge of the bacterial lawn of the new plate WORM SIDE DOWN.

20. If there was a lot of contamination, you might want to repeat steps 1 and 2 to allow the worms a second chance to crawl away from the mold.
21. Wrap your final clean plate with parafilm. You will now need to check the plate periodically until gravid nematodes develop.
22. Once there are gravid animals on the 10 cm plate, bleach the animals to clean any bacterial contaminants (Egg Drop Protocol):
    a. Make a 1:1 mixture of 1M NaOH: Bleach.
    b. Pipette ~15μL of the 1:1 mixture onto the unseeded portion of a seeded OP50 plate with an established lawn.
    c. Place a minimum of 6 gravid animals into the bleach solution (10-15 animals is good). After several minutes, the bleach should dissolve the cuticle of the adult worms, releasing the embryos.
    d. The spot will soak into the plate fairly quickly. If this occurs before most of the bacteria and adults have dissolved, add small drops of the 1:1 solution (~5μL) every couple of minutes until they are dissolved.
    e. After the bleach has soaked into the plate, move the plate, lid side down, to the optimal temperature for the worms (usually this will be 20°C)
23. After ~24 hrs, move L1s from the bleach plate to 4 separate clean, labeled 6 cm plates. You want between 10-20 L1s per plate - the more, the better.
24. Parafilm the clean plates to keep at 15°C.

Solutions used in this Recipe*:

1M NaOH (100mL yield):
There should be some of this made. Do not make this much just for this protocol.

1. Wear goggles!!
2. Measure 100mL of DI Water in a bottle
3. Add stir bar
4. Weigh 4.0g of NaOH and add it to the bottle
5. Stir until dissolved and remove the stir bar
6. Autoclave for 20-30 minutes on liquid cycle
   (Don't screw cap all the way)
7. Properly label bottle and place in storage

Egg Prep in a Drop (Spot Bleaching) Protocol

Notes: Used for synchronizing small numbers of worms and removing contamination. If you need large-scale contamination removal or many eggs for an experiment, you should likely use the Bleach Synchronizing or Bleach + NaOH Protocol.

This protocol kills all worms and laid eggs but leaves eggs in gravid worms unharmed. At 20°C, N2 *C. elegans* reach this stage in around 72-80 hours.

Time: Depends on the number of nematodes to be bleached. Assume ~1 hr.

Materials:
Bleach
M9
P25- Micropipette
Micropipette tips
OP50 seeded plates
Worm Pick
1M NaOH*
Note: You will need to mix the bleach and 1 M NaOH to form a 1:1 solution (Bleach/NaOH). Be careful not to use the 5M NaOH used in whole plate synchronizing or to inadvertently make a 2:1 (Bleach/NaOH) solution, as is done in that protocol.

Procedures:

1. Make a 1:1 mixture of 1M NaOH: Bleach.
2. Pipette ~15µL of the 1:1 mixture onto the unseeded portion of a seeded OP50 plate with an established lawn.
3. Pick 10 gravid adults off of the contaminated plate and place them directly into the 1:1 solution drop. Try to avoid bringing too much bacteria with them.
4. After several minutes, the worms should break open and release the bleach-resistant eggs.
5. The spot will soak into the plate fairly quickly. If this occurs before most of the bacteria and adults have dissolved, add small drops of the 1:1 solution (~5 µL) every couple of minutes until they are dissolved.
6. Leave the plate right side up overnight. The larvae should hatch and crawl into the OP50 lawn by the next day.
7. The next day, check that the larvae have hatched. If so, chunk out the dried-up bleach and NaOH spot to prevent contamination by any bacteria that survived.

(Continues on the next page)

~ In 24 hours, you will have synchronized L1 worms
~ In 48 hours, you will have synchronized L4 worms

Bleach Synchronizing Protocol

Notes: Used for age synchronizing multiple populations and removing contamination.
If you need small-scale contamination removal or a small number of eggs, consider using the Egg Drop Prep Protocol. This protocol kills all worms on the plate and leaves the laid eggs unharmed. If you want to remove fungal contamination, consider using the less harsh "Chunk and Go" protocol.

Time: Depends on the number of plates to be bleached. Assume ~1-2 hrs.

Materials:
Bleach
Centrifuge tube
Centrifuge
M9
OP50 seeded plates

Procedures:

1. Wash the plate with 3mL of M9. -> Refer to Worm Washing Protocol
2. Use your pipette tip to gently scrape the surface of the plate to free any eggs stuck to the plate. This can significantly increase your yield.
3. Use a micropipette tip to suck up M9 off the plate and put it into centrifuge tube.
4. Spin the centrifuge tubes at 3000rpm for 30 seconds (creates pellet)
5. Pull off the supernatant
6. Add 800 µL M9 to the centrifuge tube
7. Add 200µL of bleach
   (Steps 7 to 11 should be completed in UNDER ~TWO MINUTES)
8. Vortex for 10 seconds (spreads bleach evenly)
9. Spin the centrifuge tubes at 3000rpm for 30 seconds
10. Pull off supernatant
11. Add 2mL of M9 to centrifuge tube
12. Repeat steps 8 to 11 again. You should do two total washes.
    (Repeating the steps should also be done in ~2 minutes)
13. Plate just the pellet onto a seeded plate
    (The next day, the eggs will have hatched, and larvae will be on the lawn. At this point, you can pick transfer the larvae to a clean seeded plate with a lawn.)

~ In 24 hours, you will have synchronized L1 worms
~ In 48 hours, you will have synchronized L4 worms

Bleach and 5M NaOH Synchronizing Protocol

Notes: Used for age synchronizing and removing more intense contamination issues.
If you need small-scale contamination removal or a small number of eggs, consider using the Egg Drop Prep Protocol.

This protocol kills all worms on the plate AND most of the laid eggs. The eggs that survive are those that have not been laid and are still inside the mother.

Time: Depends on the number of plates to be bleached. Assume ~1-2 hrs.

Materials:
Bleach
Centrifuge tube
Centrifuge
Glass well
M9
5M NaOH*
OP50 seeded plates
Note: You will need to mix the bleach and the 5M NaOH together in a 2:1 solution (Bleach/NaOH). You will use 400μL of this per tube, so calculate accordingly.

Procedures:

1. Wash the plate with 3mL of M9. -> Refer to Worm Washing Protocol
2. Use a micropipette tip to suck up M9 off the plate and put it into centrifuge tube.
3. Spin the centrifuge tubes at 3000rpm for 30 seconds (creates pellet)
4. Pull off the supernatant
5. Add 1mL M9 to the centrifuge tube
6. Add 400μL of the 2:1 (Bleach: NaOH) to the tube
7. Invert the tube to mix gently (do not vortex)
8. Pull out 20μL of the solution and look under the scope using a glass well
9. Every 2 minutes, invert the tube again, pull out 10μL, and check the solution
   (The process is complete when you see that the bodies of the adult nematodes have broken open. This will vary for different worm populations.)
10. Centrifuge the tube at 1000rpm for 30 seconds
11. Wash the pellet with 2mL M9.
12. Centrifuge the tube at 1000rpm for 30 seconds
13. Remove the supernatant from the tube.

(Continues on the next page)

14. Repeat steps 12-14 two more times (3 total washes after the Bleach/NaOH step.
15. Plate only the pellet on an OP50-seeded plate.
    (The next day, the eggs will have hatched, and larvae will be on the lawn. At this point, you can pick transfer the larvae to a clean seeded plate with a lawn.)

~ In 24 hours, you will have synchronized L1 worms
~ In 48 hours, you will have synchronized L4 worms

Heat Shock Protocols

Notes: Heat shocking is used to induce a protective shock response in *C. elegans*. Depending on what you would like to do, different heat shock protocols may make more sense. Please see the method overview here to determine what is best for you.

Time: ~2-14 hrs. Multiple days in total.

Materials:
Incubator
Petri Dish (Regular or Small; 10cm or 6cm)
Paraffin film
Water Bath

Procedures (Water Bath):

25. Prior to heat shocking, age synchronize the worm populations using bleach protocols. Let them grow to the desired age before starting heat shock.
(Typically, this will be L1-L4, depends on the question being asked)
26. Set up a water bath and set it to the desired temperature (33-37 °C).
27. Place the lid on each plate and wrap a thin layer of paraffin film twice around each to seal the edges. Insecure sealing will allow water into the plate. Do not cover the bottom of the plate in film, as this can interfere with the heat transfer.
28. Submerge the plates in a circulating water bath for 1-12 hours. You can use a weight or tube rack to keep them submerged the entire time.
29. Remove the plates from the water bath, dry with a paper towel, and then remove the paraffin film. Then place the worms in a 20°C incubator to recover.
30. Protocols call for anywhere from 6-24 hours of recovery time.

Note: You can also do this with an incubator and not a water bath. Procedure below.

Procedures (Incubator):

1. Prior to heat shocking, age synchronize the worm populations using bleach protocols. Let them grow to the desired age before starting heat shock.
(Typically, this will be L1-L4, depends on the question being asked)
2. Set up an incubator and set it to the desired temperature (33-37 °C).
3. Place the lid on each plate and wrap a thin layer of paraffin film around each to seal the edges. Do not cover the bottom of the plate in film.
4. Place the plates in the incubator for 1-12 hours.
5. Remove the plates from the incubator and remove the paraffin film. Then place the worms in a 20°C incubator to recover.
6. Protocols call for anywhere from 6-24 hours of recovery time.

Generating Males via Heat Shock

Notes: Males are very rare in most wild-type strains (0.02%) and can be hard to find. If you need to use males for an experiment and they need to be in a specific genetic background, you can use this protocol to generate them.

Time: ~8 hrs.

Materials:
6cm Petri Dish (Small ones, regular is 10cm)
Plates Parafilm
Water Bath
Worm Pick

Procedures (Water Bath):

31. Set up and label 6 6cm plates. Refer to lab labeling protocols.
32. Set up a water bath and set the temperature at 31.5°C.
33. Use your pick to move 10 late L4 stage hermaphrodites onto each small plate.
34. Place the lid on each plate and wrap a thin layer of paraffin film twice around each to seal the edges. Insecure sealing will allow water into the plate. Do not cover the bottom of the plate in film, as this can interfere with the heat transfer.
35. Submerge the plates in a circulating water bath for 6 hours.
36. Remove the plates from the water bath, dry with a paper towel, and then remove the paraffin film. Then place the worms in a 20°C incubator to recover.
37. After 4-5 days, there should be males on the plate. Typically, this protocol will create 2-5% males in the F1 population.

Note: You can also do this with an incubator and not a water bath. Procedure below.

Procedures (Incubator):

1. Set up and label 6 6cm plates. Refer to lab labeling protocols.
2. Set up an incubator at 31.5°C.
3. Use your pick to move 10 late L4 stage hermaphrodites onto each small plate.
4. Place the lid on each plate and wrap a thin layer of paraffin around each to seal.
5. Place the plates in the incubator for 6 hours.
6. Remove the plates from the incubator and remove the paraffin film.
7. Place the worms in a 20°C incubator to recover.
8. After 4-5 days, there should be males on the plate. Typically, this protocol will create 2-5% males in the F1 population.

From: Michael Koelle, Yale University (http://info.med.yale.edu/mbb/koelle/)

EMS Mutagenesis Protocols

Notes: Ethyl methanesulfonate (EMS) is a mutagen and carcinogen. This is perhaps the most dangerous protocol in the lab. You should not be doing this unless you have already received permission from Dr. Lewis. EMS typically produces only point mutations via guanine alkylation and shows a G:C-to-A:T transition bias.

It is imperative to use the utmost care with this protocol. Please use all PPE and avoid using gloves after touching the EMS bottle or any of the EMS equipment

Time: ~2-14 hrs. Multiple days in total.

Materials:
Centrifuge Tube (15mL)
Cut micropipette tips (wide bore)
Ethyl methanesulfonate (EMS)
Fume Hood
M9
Micropipette
Incubator
Potassium Hydroxide (KOH)
Petri Dish (Regular or Small; 10cm or 6cm)
Paraffin film
Spinning Wheel

Procedures (Water Bath):

1. Wash worms off plates with M9, using a micropipette. You want to use plates with a lot of early L4 larvae. -> Refer to worm washing protocol.
2. After removing the supernatant for the last time in the wash protocol, fill the centrifuge tube with 3mL of M9.
3. Also, place 1mL of M9 into a separate 15mL tube.
4. While wearing gloves, <u>in the fume hood</u>, using a case of tips and a pipette that is only used for EMS, add 20µL EMS to the 15mL tube containing 1mL of M9. EMS is kept in its own special box in the chemical storage area. Keep a separate trash jar in the hood for gloves and tips that have been exposed to EMS.
5. Transfer the 3 ml of worms into the tube of EMS. Parafilm the top. Place the tube on a spinning wheel at 20°C for four hours.
   (The final concentration of EMS is 47 mM)
6. Spin the worms down at 1000rpm for 30 seconds and remove the supernatant. Place this supernatant in a waste tube. You can use the original worm wash tube.
7. Wash the worms twice with 3mL of M9 to dilute any remaining EMS. Transfer the supernatant to the same waste tube.

8. Transfer the worms, in a few drops of M9, to the edge of the bacterial lawn on a plate. Make sure you are using cut tips for all worm transfers!
9. Kill the EMS in the waste liquid from the washes by adding a few pellets of KOH. Mix a little, label the tube, and leave it in the hood for a day. Then pour IT down the sink, and discard the tube in the trash. Do not discard early or unlabeled.
10. Let the worms sit for 15-20 minutes (some wait 2 hours). Pick off the healthy looking late L4 animals (P0's). Mutagenizing too early (before much germline proliferation) will theoretically give fewer independently mutagenized genomes and give jackpots from those mutations that do occur. Mutagenizing too late will be ineffective. Some people pick late L4's to mutagenize, so that the animals are very young adults at the end of the EMS treatment. It is somewhat difficult to recognize L4's after the EMS treatment; because the animals are starved, the white crescent normally visible in the vulval region of well fed L4's is not very apparent. The "dot" that appears within the crescent at the very end of L4 is still visible in starved animals

Modified version of protocols made by Erik Andersen (3/9/2010)

Serratia Selection Plate (SSP) Mortality Assay Protocol

Notes: This is the protocol for using SSPs to evolve worms against a Serratia strain. If you are interested in assessing mortality with SSPs, see the mortality protocol. If you are interested in *Pseudomonas*, please see the Slow-Kill or Fast-Kill PSA protocols. Adapted from the Morran Lab at Emory University.

You should plan this and adapt it for your schedule based on the experiment and the days of the week you would like the longest days to fall on.

Time: 30 min to 8 hrs per day, TBD.

Materials:

Centrifuge Tube (15mL)
Cut micropipette tips (wide bore or cut)
Fume Hood
Glass Wells
Hand counter
M9
Micropipette
Incubators
Petri Dish (Regular 10cm)
Yardstick or ruler

Procedures to Start Selection:

Day 0, Friday: Chunk Worm Populations for Bleaching

1. Chunk worm populations onto OP50-seeded plates. 1 chunk plate for every 1000 worms needed is a good rule of thumb.
   (Make 2 chunks onto OP50 just in case. If it works, then only bleach from one)
2. Incubate plates at 20 °C until day 3.

Day 3, Monday: Bleach Synchronize, Inoculate Bacteria for Plates

<u>Bleach Synchronizing Worms</u>

1. Wash the worms off the chunk plate and into a centrifuge tube using 3ml M9
2. Spin the tubes @ 3000rpm for 30 sec
3. Take off the supernatant
4. Add 800ul M9 to the tube, then add 200ul household bleach
5. Invert the tube to mix and then centrifuge @3000 rpm for 30 sec
6. Remove the supernatant
7. Wash worms with 1 ml M9
8. Spin the tubes @3000 rpm for 30sec
9. Repeat steps 6-8 2x (3 total)
10. Divide the pellet onto 3 OP50-seeded plate and store in @20C for 48hrs

Inoculating Bacteria

1. For each parasite and OP50, use a loop to pick one colony into an LB tube
2. Put the tubes in the 28 °C incubator, shaking overnight

Day 4, Tuesday: Seed SSPs with Serratia

1. Put NGM plates under the hood with lids off to dry for ~20 minutes (or until they seem dry)
2. After the plates dry, trace the outside of the yardstick or ruler to draw two lines across the middle of the bottom of the plate. This will make lines ~1inch apart
3. Label the plates with parasite ID
4. Seed the SSPs
    a. Always start with OP50, then parasites
    b. Add 35ul of bacteria to the appropriate side of the plate (for OP50, you can use the same tip throughout. Switch tips between each different parasite)
    c. Use a yellow loop to spread the bacteria evenly within the 1/3 section
    d. Keep the plates flat, put them on a tray and move them into the 28 °C incubator overnight

Day 5, Wednesday: Add Worms to SSPs

Preparation

1. Pull the synchronized worm plates out of the 20 °C incubator
2. Pull the fresh SSPs out of the 28 °C incubator
3. Add 20ul of 200mg/ml Ampicillin to the middle of each SSP

Washing the Worms

8. Using a cut 1000ul tip, add 3ml M9 to wash the synchronized plates
9. Tilt the plate and remove the M9, putting it into centrifuge tube
10. Use a new tip between all worm populations
11. Once worm populations are washed into a tube, centrifuge @1000rpm for 1min
12. Remove the tubes from the centrifuge
13. Using a new, uncut tip for each tube, remove the supernatant and leave the lids open
14. Add 1 ml M9 to each tube
15. Close the lids
16. Centrifuge all the tubes @1000rpm for 1min
17. Remove the tubes
18. Repeat steps 6-10 (2 washes total)
19. Remove the supernatant and add 1ml M9

Calculating Worm Concentration

1. Invert tube to homogenize
2. Pull 3 20ul samples into the glass wells (use a new tip for each drop, the wells are not sterile and this can contaminate your worms)
3. Count the number of worms in each sample (you should aim to have 30-60 worms in each drop…any more or less will be too difficult to work with. Dilute or concentrate worms accordingly)
4. Take the average of the 3 samples and divide by 20 to get avg # worms in 1ul

5. Using this concentration, calculate the volume needed to give 200worms
6. Randomize your assay plates and 3 OP50-seeded plates (these are the control plates) by worm population
7. Homogenize your worm population, and using a new tip for each plate, add the above-calculated volume onto the middle, back portion of the parasite side of the SSP, and onto the control plates. Be sure to re-mix the worms every 2 or 3 plates.
8. Keep the plates flat, leave the lids off to dry (~10 mins), stack them onto a tray and store them in the 20 °C incubator

Day 6, Thursday: Count Control Plates (check how many worms we placed)

1. 24 hours after adding worms to the assay plates, count the number of individuals on the 3 control plates. Use the hand counter.
2. Take the average of the 3 counts and use this as the actual number plated (the denominator of the survival calculation)

Day 7, Friday: Move surviving worms to next round of selection or hold over

1. 48 hours after adding worms to assay plates, move the surviving individuals from the OP50 side of the SSP into the next round of selection. This can be done one of two ways.
    a. The first option is to use M9 to wash all surviving worms off the OP50. If using this method, use 2mL of M9 and follow the wash protocols. Be sure to not get any *Serratia* in the M9.
    b. The second option is to pick worms from the lawn directly using a pick
2. At this point, worms can be moved to a hold over plate to allow them to recover from selection and to allow population sizes to increase.
    a. This step can be cut if required but can cause more genetic drift and less repeatable results compared to hold over plate methods.

Day 10, Monday:
1. Repeat steps from the previous week. Passaging generation changes on Wednesday when worms go into the next round of selection.

Serratia Selection Plate (SSP) Mortality Assay Protocol

Notes: This is the protocol for using SSPs to score worms for morality against a Serratia strain. If you are interested in doing experimental evolution with SSPs, see the SSP experimental passaging protocol. If you are interested in *Pseudomonas*, please see the Slow-Kill or Fast-Kill PSA protocols. Adapted from the Morran Lab at Emory University.

Time: 30 min to 8 hrs per day, 7 days in total.

Materials:
Centrifuge Tube (15mL)
Cut micropipette tips (wide bore or cut)
Fume Hood
Glass Wells
Hand counter
M9
Micropipette
Incubators
Petri Dish (Regular 10cm)
Yardstick or ruler

Procedures:
Day 0, Friday: Chunk Worm Populations for Bleaching

3. Chunk worm populations onto OP50-seeded plates. 1 chunk plate for every 1000 worms needed is a good rule of thumb.
   (Make 2 chunks onto OP50 just in case. If it works, then only bleach from one)
4. Incubate plates at 20 °C until day 3.

Day 3, Monday: Bleach Synchronize, Inoculate Bacteria for Plates

Bleach Synchronizing Worms

11. Wash the worms off the chunk plate and into a centrifuge tube using 3ml M9
12. Spin the tubes @ 3000rpm for 30 sec
13. Take off the supernatant
14. Add 800ul M9 to the tube, then add 200ul household bleach
15. Invert the tube to mix and then centrifuge @3000 rpm for 30 sec
16. Remove the supernatant
17. Wash worms with 1 ml M9
18. Spin the tubes @3000 rpm for 30sec
19. Repeat steps 6-8 2x (3 total)
20. Divide the pellet onto 3 OP50-seeded plate and store in @20C for 48hrs

Inoculating Bacteria

3. For each parasite and OP50, use a loop to pick one colony into an LB tube
4. Put the tubes in the 28 °C incubator, shaking overnight

Day 4, Tuesday: Seed SSPs with Serratia

5. Put NGM plates under the hood with lids off to dry for ~20 minutes (or until they seem dry)
6. After the plates dry, trace the outside of the yardstick or ruler to draw two lines across the middle of the bottom of the plate. This will make lines ~1inch apart
7. Label the plates with parasite ID
8. Seed the SSPs
    a. Always start with OP50, then parasites
    b. Add 35ul of bacteria to the appropriate side of the plate (for OP50, you can use the same tip throughout. Switch tips between each different parasite)
    c. Use a yellow loop to spread the bacteria evenly within the 1/3 section
    d. Keep the plates flat, put them on a tray and move them into the 28 °C incubator overnight

Day 5, Wednesday: Add Worms to SSPs

<u>Preparation</u>

4. Pull the synchronized worm plates out of the 20 °C incubator
5. Pull the fresh SSPs out of the 28 °C incubator
6. Add 20ul of 200mg/ml Ampicillin to the middle of each SSP

<u>Washing the Worms</u>

20. Using a cut 1000ul tip, add 3ml M9 to wash the synchronized plates
21. Tilt the plate and remove the M9, putting it into centrifuge tube
22. Use a new tip between all worm populations
23. Once worm populations are washed into a tube, centrifuge @1000rpm for 1min
24. Remove the tubes from the centrifuge
25. Using a new, uncut tip for each tube, remove the supernatant and leave the lids open
26. Add 1 ml M9 to each tube
27. Close the lids
28. Centrifuge all the tubes @1000rpm for 1min
29. Remove the tubes
30. Repeat steps 6-10 (2 washes total)
31. Remove the supernatant and add 1ml M9

<u>Calculating Worm Concentration</u>

9. Invert tube to homogenize
10. Pull 3 20ul samples into the glass wells (use a new tip for each drop, the wells are not sterile and this can contaminate your worms)
11. Count the number of worms in each sample (you should aim to have 30-60 worms in each drop…any more or less will be too difficult to work with. Dilute or concentrate worms accordingly)
12. Take the average of the 3 samples and divide by 20 to get avg # worms in 1ul
13. Using this concentration, calculate the volume needed to give 200worms

14. Randomize your assay plates and 3 OP50-seeded plates (these are the control plates) by worm population
15. Homogenize your worm population, and using a new tip for each plate, add the above-calculated volume onto the middle, back portion of the parasite side of the SSP, and onto the control plates. Be sure to re-mix the worms every 2 or 3 plates.
16. Keep the plates flat, leave the lids off to dry (~10 mins), stack them onto a tray and store them in the 20 °C incubator

Day 6, Thursday: Count Control Plates (check how many worms we placed)

3. 24 hours after adding worms to the assay plates, count the number of individuals on the 3 control plates. Use the hand counter.
4. Take the average of the 3 counts and use this as the actual number plated (the denominator of the survival calculation)

Day 7, Friday: Score Assay Plates (Determine mortality rates)

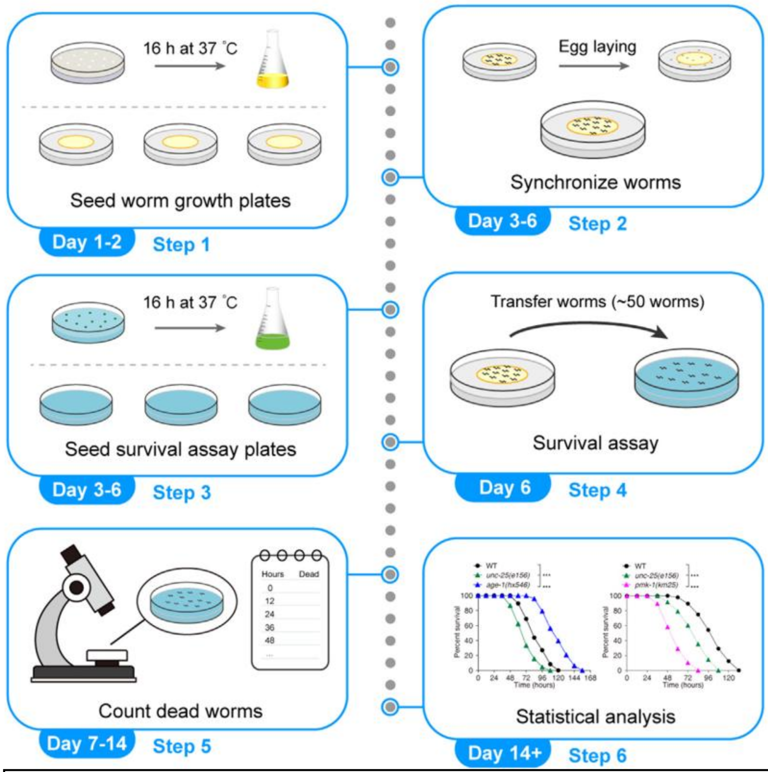
3. 48 hours after adding worms to assay plates, count the adult/L4 individuals on the OP50 side and in the middle section of the SSP. Use the hand counter. Record these numbers separately.

<u>Pseudomonas aeruginosa PA14 Slow Kill (SK) Protocol</u>

Notes: This is the protocol for assessing worm morality against a PA14. If you are interested in *PA14* Fast-Kill (toxin) protocols or *Serratia* selection, see those protocols. Adapted from " Protocol for survival assay of *Caenorhabditis elegans* to *Pseudomonas aeruginosa* PA14 infection" (https://doi.org/10.1016/j.xpro.2024.103070)

Time: Varies per day, 14 days in total.

Materials:
0.22 µm PES filter
Agar Powder
Centrifuge Tube (15mL)
Cut micropipette tips (wide bore or cut)
5-Fluoro-2'-deoxyuridine (FUdR)*
Fume Hood
Hand counter
Lauria Broth (LB)
M9
Micropipette
Nematode Growth Media (NGM)
Incubators (20C,25C,37C)
Shaking Incubator
Petri Dish (10cm and 6cm)



Overview from Cell STAR Protocols.

Bacterial Procedures:

1. Before starting the protocol, you need a freshly prepared plate of LB with PA14 streaked and another plate with OP50. Follow the instructions in the LB Media protocol and the Culturing bacteria protocol to create these. Streak plates of OP50 and PA14 can be kept at 4°C for 2 weeks and 1 week, respectively. You will also need liquid LB media. OP50 and PA14 can be incubated at 37°C for 12-16 hours to facilitate growth.
Do you best to grow the OP50 and PA14 in separate incubators
2. Prepare two bottles of 500mL NGM. This can be done from scratch, NGM lite powder or from NGM powder. Regardless, Slow Kill (SK) plates have an identifical forumula as nromal NGM besides the addition of one extra gram of bacto-peptone. Adjust the recipies accordingly (add 0.5g extra of bactopeptone to each 500mL solution). Autoclave like normal then remove.
    a. From the first 500mL bottle, pour media into assorted 60mm plates.
    b. For the second bottle, add 5-Fluoro-20-deoxyuridine (FUdR) at a concentration of 50 µL/mL. For 500mL of media, add 1mL of the labs 25mg/mL stock solution. Then pour into 35mm Plates.
    c. SK and NGM plates can be stored at 4°C for up to a month before use.

This denotes the end of the “prep” steps

3. Inoculate 100mL of LB media in a 250mL flask with a single colony of E. coli OP50 from the fresh OP50 streak plate. Incubate at 37°C for 16 hours, shaking at 200rpm. This will be used to seed NGM plates.
4. Seed 250µL of the OP50 culture in the center of a 60mm NGM plate and spread it by shaking the plate.
    a. Dry the OP50 lawn in the hood at 22-25°C for 6-8 hours.
    b. Grow the lawn on the NGM plate upside down at 37°C for 24-36hrs.
    Do not let the lawn reach the edge of the dish
5. Inoculate 10mL of LB media in a sterile test tube with a colony of PA14. Incubate at 37°C for 12-16 hours, shaking at 200rpm.
   DO NOT ALLOW TO GROW FOR MORE THAN 16 HOURS at 37°C
6. Seed 150 µL of fresh PA14 on the 35 mm NGM + FUdR plates and swirl to ensure that the PA14 culture covers the full surface of plate.
   Dry the PA14 full-lawn plates for about 20 min in the laminar hood at 22-25°C. Incubate these plates at 37°C for 24 h. Move the plates to another incubator and incubate at 25°C for 24 h. Place the plates on a clean bench at 22-25°C for 1 h before the survival assay.
   If separate hoods cannot be used for the OP50 and PA14, please disinfect all materials and surfaces between bacterial manipulations.

Nematode Procedures:

1. Two days before the start of assays, bleach-NaOH worms to synchronize their ages.
    a. For small numbers of worms, use the egg drop recipe
    b. For larger numbers, bleach the entire plate
2. Wash the plate with 3mL of M9. -> Refer to Worm Washing Protocol
3. Use a micropipette tip to suck up M9 off the plate and put it into centrifuge tube.
4. Spin the centrifuge tubes at 3000rpm for 30 seconds (creates pellet)
5. Pull off the supernatant
6. Add 1mL M9 to the centrifuge tube
7. Add 400µL of the 2:1 (Bleach: NaOH) to the tube (5M NaOH not 1M)
8. Mix gently, then pull out 10µL, and check the solution
   (The process is complete when you see that the bodies of the adult nematodes have broken open. This will vary for different worm populations.)
    a. Adult and larval cuticles will continue to dissolve, but the unhatched progeny are partially protected from the bleach by their eggshells. Shake periodically. Within 4–5 min, most, but not all, of the adult worms should dissolve. Do not allow the reaction to continue for more than 7 min, or the eggs will begin to die.
9. Quickly add 10mL of M9 buffer to dilute the bleach. Centrifuge the tube at 1000rpm for 30 seconds and remove the supernatant.
10. Wash the pellet with 5mL M9.
11. Centrifuge the tube at 1000rpm for 30 seconds
12. Remove the supernatant from the tube.

13. Wash the pellet with 5mL M9.
14. Centrifuge the tube at 1000rpm for 30 seconds
15. Remove the supernatant from the tube.
16. After the last wash, plate the pellet directly outside of the Bacterial lawn on a seeded OP50 plate. Allow nematodes to develop for 40-48 hours at 20°C. This should give you age synchronization at the L4 stage.
17. To begin assays, add nematodes to SK plates after they have been removed from the 25°C incubator. Add 50 individuals of each tester strain to their own set of 3 plates. This gives the treatment replication. Nematodes can be moved via picking or concentration counting. If you are doing several strains, concentration counting may be the best option.
    a. For picking, simply move 50 nematodes as quickly as possible.
    b. For concentration counts, use 3mL of M9 to wash the plate of L4 synchronized nematodes into a 15mL conical tube. Each tester strain should receive its own tube and be kept separated in treatment.
    c. After the nematodes are in the tube, give it a gentle mix and then remove three 20μL aliquots of the sample and place them into a 3-well glass.
    d. Count nematodes across the three plates, average them, and then divide by 20 to determine the concentration of nematodes per 1mL of M9.
    e. EACH TUBE NEEDS ITS OWN CONCENTRATION COUNT
18. Add the number of mL needed to place 50 nematodes on each plate and incubate at 25°C to start the survival assay.
19. Score worms every 12 hours for 72 hours (6 counts).
    a. Worms are scored as dead if they fail to respond when the nose and the tail of worms are touched by an eyebrow worm picker three times. Carefully watch for worm movement. If dead, pick it out of the survival assay plate and score it dead.

Calendar on the next page **

SK Schedule by Event:

Monday	Tuesday	Wednesday	Thursday	Friday	Saturday	Sunday
In these days you should: 1. Make sure you have a fresh streak plate with PA14 and OP50. 2. Make NGM and SK media plates on 6cm and 3.5cm petri dishes, respectively. 3. Double check worm populations				Pre-bleach chunk (gravid adults in ~72hrs)	FREE DAY	Incubate liquid media with PA14 and OP50 if needed.
Spread PA14 lawns and Incubate SK plates at 37°C. Beach nematodes	Incubate SK plates at 25°C	Seed SK plates with nematodes	Conduct control, 12hr, and 24 hr count protocols	Conduct 36- and 48-hour count protocols	Conduct 60 and 72-hour count protocols	Compile data and prepare an Excel file
Compile data and prepare an Excel file	Data and statistics	Data and statistics	Data and statistics			

Pseudomonas aeruginosa PA14 Fast Kill (FK) Protocol

Notes: This protocol is for assessing worm mortality against a PA14. It relies on toxin-mediated killing. If you are interested in *PA14* Slow-Kill (Gut) protocols or *Serratia* selection, see those protocols. Adapted from protocols by Erik Andersen (2008).

Time: Varies per day, 14 days in total.

Materials:
Agar 8.5g
Bacto-Peptone 5g
1M Calcium Chloride (CaCl2) 0.5mL*
1M Magnesium Sulfate (MgSO4) 0.5mL*
1M Potassium Phosphate (KH2PO4) 12.5 mL*
Sodium Chloride (NaCl) 5g
Cholesterol in Ethanol (5mg/mL) 0.5mL*
20% Glycerol 50mL
1M Sorbitol 25mL
DI water
Aluminum Foil
Autoclave tape
Balance/Scale
Heat-Resistant gloves
Nalgene Pan (Autoclave pan)
Petri Dishes (plates) of desired size
P-1000 Micropipette

Bacterial Procedures:

7. Before starting the protocol, you need a freshly prepared plate of LB with PA14 streaked and another plate with OP50. Follow the instructions in the LB Media protocol and the Culturing bacteria protocol to create these. Streak plates of OP50 and PA14 can be kept at 4°C for 2 weeks and 1 week, respectively. You will also need liquid LB media. OP50 and PA14 can be incubated at 37°C for 12-16 hours to facilitate growth.
   Do you best to grow the OP50 and PA14 in separate incubators
8. Prepare two bottles of 500mL NGM. This can be done from scratch, NGM lite powder or from NGM powder. Regardless, Slow Kill (SK) plates have an identifical forumula as nromal NGM besides the addition of one extra gram of bacto-peptone. Adjust the recipes accordingly (add 0.5g extra of Bacto Peptone to each 500mL solution). Autoclave like normal then remove.
   a. From the first 500mL bottle, pour media into assorted 60mm plates.
   b. For the second bottle, add 5-Fluoro-20-deoxyuridine (FUdR) at a concentration of 50 µL/mL. For 500mL of media, add 1mL of the labs 25mg/mL stock solution. Then pour into 35mm Plates.

   c. SK and NGM plates can be stored at 4°C for up to a month before use.

This denotes the end of the “prep” steps

9. Inoculate 100mL of LB media in a 250mL flask with a single colony of E. coli OP50 from the fresh OP50 streak plate. Incubate at 37°C for 16 hours, shaking at 200rpm. This will be used to seed NGM plates.
10. Seed 250µL of the OP50 culture in the center of a 60mm NGM plate and spread it by shaking the plate.
    a. Dry the OP50 lawn in the hood at 22-25°C for 6-8 hours.
    b. Grow the lawn on the NGM plate upside down at 37°C for 24-36hrs.
       Do not let the lawn reach the edge of the dish
11. Inoculate 10mL of LB media in a sterile test tube with a colony of PA14. Incubate at 37°C for 12-16 hours, shaking at 200rpm.
    DO NOT ALLOW TO GROW FOR MORE THAN 16 HOURS at 37°C
12. Seed 150 µL of fresh PA14 on the 35 mm NGM + FUdR plates and swirl to ensure that the PA14 culture covers the full surface of plate.
    Dry the PA14 full-lawn plates for about 20 min in the laminar hood at 22-25°C. Incubate these plates at 37°C for 24 h. Move the plates to another incubator and incubate at 25°C for 24 h. Place the plates on a clean bench at 22-25°C for 1 h before the survival assay.
    If separate hoods cannot be used for the OP50 and PA14, please disinfect all materials and surfaces between bacterial manipulationsPseudomonas.

Nematode Procedures:

1. Two days before the start of assays, bleach-NaOH worms to synchronize their ages.
   a. For small numbers of worms, use the egg drop recipe
   b. For larger numbers, bleach the entire plate
2. Wash the plate with 3mL of M9. -> Refer to Worm Washing Protocol
3. Use a micropipette tip to suck up M9 off the plate and put it into centrifuge tube.
4. Spin the centrifuge tubes at 3000rpm for 30 seconds (creates pellet)
5. Pull off the supernatant
6. Add 1mL M9 to the centrifuge tube
7. Add 400µL of the 2:1 (Bleach: NaOH) to the tube (5M NaOH not 1M)
8. Mix gently, then pull out 10µL, and check the solution
   (The process is complete when you see that the bodies of the adult nematodes have broken open. This will vary for different worm populations.)
   a. Adult and larval cuticles will continue to dissolve, but the unhatched progeny are partially protected from the bleach by their eggshells. Shake periodically. Within 4–5 min, most, but not all, of the adult worms should dissolve. Do not allow the reaction to continue for more than 7 min, or the eggs will begin to die.
9. Quickly add 10mL of M9 buffer to dilute the bleach. Centrifuge the tube at 1000rpm for 30 seconds and remove the supernatant.
10. Wash the pellet with 5mL M9.
11. Centrifuge the tube at 1000rpm for 30 seconds

12. Remove the supernatant from the tube.
13. Wash the pellet with 5mL M9.
14. Centrifuge the tube at 1000rpm for 30 seconds
15. Remove the supernatant from the tube.
16. After the last wash, plate the pellet directly outside of the Bacterial lawn on a seeded OP50 plate. Allow nematodes to develop for 40-48 hours at 20°C. This should give you age synchronization at the L4 stage.
17. To begin assays, add nematodes to SK plates after they have been removed from the 25°C incubator. Add 50 individuals of each tester strain to their own set of 3 plates. This gives the treatment replication. Nematodes can be moved via picking or concentration counting. If you are doing several strains, concentration counting may be the best option.
    a. For picking, simply move 50 nematodes as quickly as possible.
    b. For concentration counts, use 3mL of M9 to wash the plate of L4 synchronized nematodes into a 15mL conical tube. Each tester strain should receive its own tube and be kept separated in treatment.
    c. After the nematodes are in the tube, give it a gentle mix and then remove three 20µL aliquots of the sample and place them into a 3-well glass.
    d. Count nematodes across the three plates, average them, and then divide by 20 to determine the concentration of nematodes per 1mL of M9.
    e. EACH TUBE NEEDS ITS OWN CONCENTRATION COUNT
18. Add the number of mL needed to place 50 nematodes on each plate and incubate at 25°C to start the survival assay.
19. Score worms every 12 hours for 72 hours (6 counts).
    a. Worms are scored as dead if they fail to respond when the nose and the tail of worms are touched by an eyebrow worm picker three times. Carefully watch for worm movement. If dead, pick it out of the survival assay plate and score it dead.

Calendar on the next page **

FK Schedule by Event:

Monday	Tuesday	Wednesday	Thursday	Friday	Saturday	Sunday
In these days you should: 1. Make sure you have a fresh streak plate with PA14 and OP50. 2. Make NGM and SK media plates on 6cm and 3.5cm petri dishes, respectively. 3. Double check worm populations				Pre-bleach chunk (gravid adults in ~72hrs)	FREE DAY	Incubate liquid media with PA14 and OP50 if needed.
Spread PA14 lawns and Incubate SK plates at 37°C. Beach nematodes	Incubate SK plates at 25°C	Seed SK plates with nematodes	Conduct control, 12hr, and 24 hr count protocols	Conduct 36- and 48-hour count protocols	Conduct 60 and 72-hour count protocols	Compile data and prepare an Excel file
Compile data and prepare an Excel file	Data and statistics	Data and statistics	Data and statistics			

Media Solutions and Recipes

Freezing Solution Recipe

Notes: 1L Yield. Used to keep nematodes in -80°C storage.

Time: 1.75 hours, 45 minutes prep + ~1 hour in autoclave

Materials:
DI water
Glycerol 300g
0.1M Magnesium Sulfate ($MgSO_4$)*
Sodium Chloride (NaCl) 5.85g
1 M Sodium Hydroxide (NaOH) 5.6mL*
Potassium Phosphate (KH_2PO_4) 6.8g
Balance/Scale
Erlenmeyer flask(s)
Graduated Cylinder
Micropipette(s)
Micropipette tips
Stir Bar + Magnetic stir bar retriever
Stir Plate
Weigh Boats
Notes: Recipes for aqueous chemical solutions on the second page.

Media Mixing Procedure (Pre-Autoclave):

1. REMEMBER TO WEAR GLOVES, THESE CHEMICALS CAN HARM YOU!!!!
   After putting on proper PPE, place the following in the graduated cylinder
   A. NaCl 5.85g
   B. KH_2PO_4 6.8g
      (You can use the same weigh boat in sequence for all materials)
   C. Glycerol 300g
      (When weighing glycerol, weigh 150g in the weigh boat twice)
   D. 1M NaOH 5.6mL
      (If aqueous solution is not ready, equivalent mass of NaOH is 0.224g)
   E. Then, add DI water until the total volume reaches 1L
2. Add stir bar to graduated cylinder, place on hotplate, and activate
3. Once dissolved, remove the stir bar using the stir bar retriever
4. Divide the mixture into two 1L Erlenmeyer flasks (500mL per) or 1 2L flask
5. At the same time, autoclave six 250mL bottles in a separate pan
   (Alternatively, just make sure you have six sterile 250mL bottles)

(Instructions continue on the next page)

Media Mixing Procedure (Post-Autoclave):

1. Add 3mL of 0.1M $MgSO_4$ to each flask
   (If aqueous solution is not ready, equivalent mass of $MgSO_4$ is 0.0361g)
2. Dispense into the autoclaved bottles
3. Place into the proper storage cabinet

Solutions used in this Recipe*:

1M NaOH (100mL yield)

8. Wear goggles!!
9. Measure 100mL of DI Water in a bottle
10. Add stir bar
11. Weigh 4.0g of NaOH and add it to the bottle
12. Stir until dissolved and remove the stir bar
13. Add autoclave tape and autoclave for 20-30 minutes on liquid cycle
    (Don't screw cap all the way)
14. Properly label bottle and place in storage

0.1M $MgSO_4$ (100mL yield)

1. Wear goggles!!
2. Measure 100mL of DI water in a bottle
3. Add stir bar
4. Weigh 1.204g of $MgSO_4$ and add it to the bottle
5. Stir until dissolved and remove the stir bar
6. Add autoclave tape and autoclave for 20-30 minutes on liquid cycle
   (Don't screw cap all the way)
7. Properly label bottle and place in storage

Lauria Broth (LB) Media and Plate Recipe

Notes: 1L Yield. Used as a medium for bacterial growth.

Time: 1.5 hours, 30 minutes prep + ~1 hour in autoclave

Materials:
Agar 15g (only if making plates)
DI water 1L
LB Powder 25g
Sodium Chloride (NaCl) 5g
Tryptone 10g
Yeast Extract 5g
Balance/Scale
Graduated Cylinder
Micropipette(s)
Micropipette tips
Stir Plate (optional)
Small bottles
Stir Bar + Magnetic stir bar retriever (optional)
Weigh Boats

From Premade LB Powder:

1. Measure 1L of DI H20 and add it to a flask.
   (The volume of the flask should always be around double the volume being prepared for the autoclave. If you do not have a 2L flask available, use two 1L flasks or two 1L bottles.)
2. Weigh out 25g of LB powder and add it to the flask. Give it a vigorous swirl. (Optionally, you can use a stir bar and a stir plate.)
3. If you intend to make plates and not liquid broth, this is the point where you will add 15g of Agar powder (this allows it to solidify once cooled).
4. Mix vigorously or use a stir bar until dissolved. Remove the bar if one was used.
5. Cover with aluminum foil and add autoclave tape.
6. Place in the autoclave pan with ½ inch of water and autoclave on the liquid cycle for 20-30 minutes.
7. After the cycle, remove using heat-resistant gloves
8. Allow to cool, then tighten bottles and store

From Scratch:

1. Measure 1L of DI H20 and add it to a flask or graduated cylinder.
   (The volume of the flask should always be around double the volume being prepared for the autoclave. If you do not have a 2L flask available, use two 1L flasks or two 1L bottles.)

2. REMEMBER TO WEAR GLOVES, THESE CHEMICALS CAN HARM YOU!!!!
   After putting on proper PPE, place the following in the graduated cylinder:
   A. Tryptone 10g
   B. Yeast extract 5g
   C. NaCl 10g
   D. Agar (15g) if making solid plates
6. Add stir bar to graduated cylinder or flask, place on hot plate, and activate.
7. Once dissolved, remove the stir bar and dispense into smaller bottles
   (Do not screw cap all the way)
8. Add autoclave tape
9. Place in the autoclave pan with ½ inch of water
10. After the cycle, remove using heat-resistant gloves
11. Allow to cool, then tighten bottles and store

Note: Antibiotics can also be added to these plates. Refer to the antibiotic protocols on the NGM plate protocol

M9 Solution Recipe

Notes: 1L Yield. Used to move nematodes between plates.

Time: 1.5 hours, 30 minutes prep + ~1 hour in autoclave

Materials:
DI water
1M Magnesium Sulfate ($MgSO_4$)* 1mL
Sodium Chloride (NaCl) 5g
Sodium Phosphate (Na_2HPO_4) 6g
Potassium Phosphate (KH_2PO_4) 3g
Balance/Scale
Graduated Cylinder
Micropipette(s)
Micropipette tips
Stir Plate
Small bottles
Stir Bar + Magnetic stir bar retriever
Weigh Boats
Notes: Recipes for aqueous chemical solutions on the second page.

Media Mixing Procedure (Pre-Autoclave):

12. REMEMBER TO WEAR GLOVES, THESE CHEMICALS CAN HARM YOU!!!!
    After putting on proper PPE, place the following in the graduated cylinder:
    A. KH_2PO_4 3g
    B. Na_2HPO_4 6g
    C. NaCl 5g
    D. 1M $MgSO_4$ 1mL
    E. Then, add DI water until the total volume reaches 1L
13. Add stir bar to graduated cylinder, place on hot plate, and activate
14. Once dissolved, remove the stir bar and dispense into smaller bottles
    (Do not screw cap all the way)
15. Add autoclave tape
16. Place in the autoclave pan with ½ inch of water
17. After the cycle, remove using heat-resistant gloves
18. Allow to cool, then tighten bottles and store

(Solutions used on the next page)

Solutions used in this Recipe*:

1M $MgSO_4$ (100mL yield)

8. Wear goggles!!
9. Measure 100mL of DI water in a bottle
10. Add stir bar
11. Weigh 12.0367g of $MgSO_4$ and add it to the bottle
12. Stir until dissolved and remove the stir bar
13. Add autoclave tape and autoclave for 20-30 minutes on liquid cycle (Don’t screw cap all the way)
14. Properly label bottle and place in storage

Nematode Growth Media Lite (NGM Lite) Plate Recipe

Notes: Used to rear worms. Makes 500mL worth of liquid media or ~20 plates.

Note, this protocol is for NGM Lite, not normal NGM powder. If you do not have NGM Lite, please see the NGM from Scratch or NGM (Normal) protocol. These are the same, but the required preparation steps are distinct.

Time: ~2.25 Hours, 1.25 hours prep and pour + 1 hour in autoclave

Materials:
14.5 g NGM Lite
500mL DI water
Aluminum Foil
Autoclave tape
Balance/Scale
Heat-Resistant gloves
Nalgene Pan (Autoclave pan)
Petri Dishes (plates) of desired size
Serological Pipette Gun & 50mL serological pipettes (recommended for small plates)
P-1000 Micropipette (antibiotic plates)

Media Mixing Procedure:

1. Add 14.5g of NGM Lite powder with 500mL dH2O in a 1L flask
   (Total flask volume should always be ≥ 2x volume of media)
2. Cover with foil, then place autoclave tape over foil.
3. Swirl the mixture and place it in the autoclave pan with ½ inch of water
4. Autoclave 30 minutes on liquid cycle
   (Remember to wear heat-resistant gloves when taking the pan out of autoclave)
5. Swirl immediately after the cycle and let stand for 10 minutes before pouring
6. Give one final swirl before pouring to ensure equal mixing

Plate Pouring Procedure:

A. Large plates (100mm)
   1. Set up your plates: you can stack up to 5 at a time
   2. Pour just enough NGM to cover the bottom of the plate (~200µL per)
      (If you stacked, start at the bottom-most plate and work up)
   3. Let the plates cool and solidify before moving to storage (~2 hours)

B. Small Plates (x < 100mm)
   1. Set up your plates: you can stack 3-4 at a time
      (These turn over much easier than large plates)
   2. Insert a 50 mL serological pipette into the Serological pipette gun
      (Top button sucks up liquid, bottom button ejects liquid)

3. Place the tip of the pipette into the NGM & suck up 50 µL at a time
   (Try not to exceed 50mL because it can go into the gun ☹)
4. Eject ~5mL of NGM into each plate
   (Be careful when ejecting, liquid ejects very quickly and can cause overfills)
   (DO NOT LET THE SEROLOGICAL PIPET TOUCH ANYTHING THAT IS NOT STERILE)
5. Let the plates cool and solidify before moving to storage (~2 hours)

C. Antibiotic Plates

1. Once the media is cool enough to handle (~15 minutes), use a P-1000 micropipette to add antibiotics to the flask of media
2. The concentration of antibiotics needed will determine the volume added.
   (The chart below assumes an antibiotic aliquot concentration of 100mg/mL)
3. Let the plates cool and solidify before moving to storage (~2 hours)

Desired plate antibiotic concentration	Volume of 100mg/mL to be added to 500mL
50 µg/ mL	250 µL
100 µg/ mL	500 µL
200 µg/ mL	1000 µL

Nematode Growth Media REGULAR (NGM) Plate Recipe

Notes: Used to rear worms. The recipes below are for 0.5L of liquid media or ~20 plates.

***Note, this protocol is for NGM, not NGM Lite. If you have NGM Lite, please see the NGM Lite protocol. These are the same, but the required preparation steps are distinct.

Time: ~2.5 Hours, 1.5 hours prep and pour + 1 hour in autoclave

Materials:
NGM Powder 11.5g
1M Calcium Chloride ($CaCl_2$) 0.5mL*
1M Magnesium Sulfate ($MgSO_4$) 0.5mL*
1M Potassium Phosphate (KH_2PO_4) 12.5 mL*
DI water
Aluminum Foil
Autoclave tape
Balance/Scale
Heat-Resistant gloves
Nalgene Pan (Autoclave pan)
Petri Dishes (plates) of desired size
P-1000 Micropipette

Media Mixing Procedure (Pre-Autoclave):

1. Add 11.5g of NGM powder with 490 mL dH_2O in a 1L flask
   (Total flask volume should always be ≥ 2x volume of media)
2. Cover with foil, then place autoclave tape over foil.
3. Swirl the mixture and place it in the autoclave pan with ½ inch of water
4. Autoclave 30 minutes on liquid cycle
   (Remember to wear heat-resistant gloves when taking the pan out of autoclave)
5. Swirl immediately after the cycle and let stand for 10 minutes before pouring
6. Give one final swirl before pouring to ensure equal mixing

Media Mixing Procedure (Post-Autoclave):

1. Solution needs to completely cool to the desired temperature (58 °C). If the medium is not properly cooled when the $CaCl_2$, $MgSO_4$ and K_2HPO_4 are added, crystals will form in the agar. (Do not use a thermometer, use the water bath)
2. Use a micropipette tip to add 0.5 mL of 1M $CaCl_2$, then gently swirl. Discard tip.
3. Use a micropipette tip to add 12.5mL of 1M KH_2PO_4, then swirl. Discard tip.
4. Use a micropipette tip to add 0.5 mL of 1M $MgSO_4$, then gently swirl. Discard tip.

Plate Pouring Procedure follows the same protocols as the NGM Lite Protocol found earlier in this manual.

Nematode Growth Media from Scratch (NGM) Plate Recipe

Notes: Used to rear worms. The recipes below are for 0.5L of liquid media or ~20 plates.
***Note, this protocol is for making NGM from scratch, not from any premade complex media. If you have NGM Lite or NGM regular, please see those protocols.

Time: ~3 Hours, 2 hours prep and pour + 1 hour in autoclave

Materials:
Agar 8.5g
Bacto-Peptone 1.25g
1M Calcium Chloride ($CaCl_2$) 0.5mL*
1M Magnesium Sulfate ($MgSO_4$) 0.5mL*
1M Potassium Phosphate (KH_2PO_4) 12.5 mL*
Sodium Chloride (NaCl) 1.5g
Cholesterol in Ethanol (5mg/mL) 0.5mL*
DI water
Aluminum Foil
Autoclave tape
Balance/Scale
Heat-Resistant gloves
Nalgene Pan (Autoclave pan)
Petri Dishes (plates) of desired size
P-1000 Micropipette

Media Mixing Procedure (Pre-Autoclave):

1. Add 8.5g of Agar, 1.25g of Bacto-peptone, and 1.5g of sodium chloride with 500 mL dH_2O in a 1L flask (Total flask volume should always be ≥ 2x volume of media)
2. Cover with foil, then place autoclave tape over foil.
3. Swirl the mixture and place it in the autoclave pan with ½ inch of water
4. Autoclave 30 minutes on liquid cycle
   (Remember to wear heat-resistant gloves when taking the pan out of autoclave)
5. Swirl immediately after the cycle and let stand for 10 minutes before pouring
6. Give one final swirl before pouring to ensure equal mixing

Media Mixing Procedure (Post-Autoclave):

5. Solution needs to completely cool to the desired temperature (55 °C). If the medium is not properly cooled when the $CaCl_2$, $MgSO_4$ and K_2HPO_4 are added, crystals will form in the agar. (Do not use a thermometer, use the water bath)
6. Use a micropipette tip to add 0.5 mL of 1M $CaCl_2$, then gently swirl. Discard tip.
7. Use a micropipette tip to add 12.5mL of 1M KH_2PO_4, then swirl. Discard tip.
8. Use a micropipette tip to add 0.5 mL of 1M $MgSO_4$, then gently swirl. Discard tip.
9. Use a micropipette tip to add 0.5mL of 5 mg/mL Cholesterol in Ethanol, gently swirl and then discard the pipette tip.
   Plate Pouring Procedure follows the same protocols as the NGM Lite Protocol found earlier in this manual.

Chemical Solutions and Buffers

Antibiotic Stock Protocol

Notes: General protocol for antibiotics, double-check against manufacturer instructions.

Time: 30 minutes

Materials:
Antibiotic powder
Magnetic stir bar + retriever
Microtubes
Stir plate
Syringe
0.22µm Syringe filter
Solvent: Either DI water or Ethanol (100% or 70%)

Procedures:

1. Dissolve 1g of antibiotic in 10mL of solvent
2. Add stir bar and mix until dissolved
3. Remove stir bar with magnetic retriever
4. If the solvent is DI Water, then:
    a. Suck up the entire 10mL volume with the syringe
    b. Attach the syringe to the filter
    (Do not touch the filter; this will make it unsterile)
5. Place 1mL of antibiotic into microtubes
6. Store in regular refrigerator

Some antibiotics we use in the lab

Antibiotic	Solvent
Ampicillin	H20
Kanamycin	H20
Tetracycline	70% Ethanol
Chloramphenicol	100% Ethanol
Streptomycin	H20

Other antibiotic concentrations:

Concentration	Amount antibiotic	Volume Solvent
100mg/mL	1g	10mL
200mg/mL	2g	10mL
400mg/mL	4g	10mL

1M $CaCl_2$ (100mL yield)

1. Wear goggles!!
2. Measure 80mL of DI water in a bottle
3. Add stir bar
4. Weigh 11.1g of $CaCl_2$ and add it to the bottle
5. Top off with DI water until 100g
6. Stir until dissolved and remove the stir bar
7. Add autoclave tape and autoclave for 20-30 minutes on liquid cycle (Don't screw cap all the way)
8. Properly label bottle and place in storage

0.1M $MgSO_4$ (100mL yield)

1. Wear goggles!!
2. Measure 100mL of DI water in a bottle
3. Add stir bar
4. Weigh 1.204g of $MgSO_4$ and add it to the bottle
5. Stir until dissolved and remove the stir bar
6. Add autoclave tape and autoclave for 20-30 minutes on liquid cycle (Don't screw cap all the way)
7. Properly label bottle and place in storage

1M $MgSO_4$ (100mL yield)

1. Wear goggles!!
2. Measure 80mL of DI water in a bottle
3. Add stir bar
4. Weigh 12.0367g of $MgSO_4$ and add it to the bottle
5. Top off with DI water until 100g
6. Stir until dissolved and remove the stir bar
7. Add autoclave tape and autoclave for 20-30 minutes on liquid cycle (Don't screw cap all the way)
8. Properly label bottle and place in storage

1M NaOH (100mL yield)

1. Wear goggles!!
2. Measure 100mL of DI Water in a bottle
3. Add stir bar
4. Weigh 4.0g of NaOH and add it to the bottle
5. Stir until dissolved and remove the stir bar

6. Add autoclave tape and autoclave for 20-30 minutes on liquid cycle (Don’t screw cap all the way)
7. Properly label bottle and place in storage

5M NaOH (100mL yield)

1. Wear goggles!!
2. Measure 100mL of DI Water in a bottle
3. Add stir bar
4. Weigh 20.0g of NaOH and add it to the bottle
5. Stir until dissolved and remove the stir bar
6. Add autoclave tape and autoclave for 20-30 minutes on liquid cycle (Don’t screw cap all the way)
7. Properly label bottle and place in storage

20% Glucose Solution

1. Wear goggles!!
2. Measure 10mL of DI water in a flask
3. Add stir bar
4. Weigh 250mg of FUdR and add it to the flask
5. Stir until dissolved and remove the stir bar
6. Do not Autoclave!!
7. Use a syringe and a 0.22 µm PES filter to dispense 1mL of solution into centrifuge tubes or other storage tube.
8. Store at -20°C for up to 2 weeks.

50% Glycerol Solution

1. Measure 250mL of pure glycerol and add it to the bottle
2. Measure 250mL of DI water and add it to the bottle
3. Cap and shake vigorously until well mixed. Be sure the outside of the bottle is DRY before shaking. Wet bottles can slip from hands easily.
4. Add autoclave tape and autoclave for 20-30 minutes on liquid cycle (Don’t screw cap all the way)
5. Properly label the bottle and place in storage

1.5M Sorbitol Solution

1. Wear goggles!!
2. Measure 10mL of DI water in a flask

3. Add stir bar
4. Weigh 250mg of FUdR and add it to the flask
5. Stir until dissolved and remove the stir bar
6. Do not Autoclave!!
7. Use a syringe and a 0.22 μm PES filter to dispense 1mL of solution into centrifuge tubes or other storage tube.
8. Store at -20°C for up to 2 weeks.

5-Fluoro-20-deoxyuridine (FUdR) (10mL yield at 25mg/mL)

1 mL of 25mg/mL solution in 500mL of NGM gives a final concentration of 50 μL/mL

1. Wear goggles!!
2. Measure 10mL of DI water in a flask
3. Add stir bar
4. Weigh 250mg of FUdR and add it to the flask
5. Stir until dissolved and remove the stir bar
6. Do not Autoclave!!
7. Use a syringe and a 0.22 μm PES filter to dispense 1mL of solution into centrifuge tubes or other storage tube.
8. Store at -20°C for up to 2 weeks.

5 mg/mL Cholesterol in Ethanol

1. Wear goggles!
2. Measure 100mL of 100% ethanol in a bottle
3. Weigh 500mg or 0.5g of powdered cholesterol
4. Cap and shake to mix evenly. DO NOT AUTOCLAVE.
5. Properly label and place in storage.

1M KH_2PO_4 (100mL yield)

1. Wear goggles!!
2. Measure 80mL of DI water in a bottle
3. Add stir bar
4. Weigh 13.61g of KH_2PO_4 and add it to the bottle
   (Note if you have K_3PO_4 or K_2HPO_4 pellets, the amount will change)
   (K_3PO_4 = 21.23g | K_2HPO_4 = 17.42g)
5. Top off with DI water until 100g
6. Stir until dissolved and remove the stir bar
7. Add autoclave tape and autoclave for 20-30 minutes on liquid cycle
   (Don't screw cap all the way)
8. Properly label bottle and place in storage