Worm Life Cycle

**Table I**
Growth Parameters of the *Caenorhabditis elegans* Life Cycle

<table>
<thead>
<tr>
<th>Temperature (°C)</th>
<th>Embryogenesis (h)</th>
<th>Molts (h posthatch)</th>
<th>First eggs laid (h posthatch)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>L1–L2</td>
<td>L2–L3</td>
</tr>
<tr>
<td>16</td>
<td>25*</td>
<td>24</td>
<td>39</td>
</tr>
<tr>
<td>20</td>
<td>18*</td>
<td>15</td>
<td>24</td>
</tr>
<tr>
<td>25</td>
<td>14</td>
<td>11.5</td>
<td>18.5</td>
</tr>
</tbody>
</table>

* Based on Hirsh et al. (1976, Fig. 2).

b Calculated by multiplying 25°C embryogenesis time by 20 and 16°C growth rate factors of 1.3 and 2.1, respectively.

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

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. Someone in the lab should have an N2 mating plate of males, so ask around. Sometimes you may see more males in a strain during stressful conditions such as starvation or heat shock. See page 12 for protocols on generating 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).
Making 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.](image1)

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.](image2)

Morimoto lab addition:

NOTE: Make sure you label your pick! It is very easy to lose it in the worm room and people will ‘adopt’ picks that look abandoned. Going along with that, please do not use someone else’s pick without their permission!
Picking Worms
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

![Figure 3. Moving worms](image)

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.
**Chunking Worms**

From WormBook: The Online Review of *C. elegans* Biology. Maintenance of *C. elegans*

A quick and convenient method is "chunking", wherein a sterilized scalpel or spatula is used to move a chunk of agar from an old plate to a fresh plate. There will usually be hundreds of worms in the chunk of agar. The worms will crawl out of the chunk and spread out onto the bacterial lawn of the new plate. This method works well for transferring worms that have burrowed into the agar or are difficult to pick individually (such as on a starved plate). The chunking method is fine for transferring homozygous stocks but it is not advisable if the population is heterozygous or if a stock must be maintained by mating.
NGM Plates

In the Morimoto lab, people generally use the 6 cm plates for maintaining strains and a variety of experiments, while the 3.5 and 10 cm plates are used for a few specific things. Because we go through so many 6 cm plates, there are generally a couple boxes of common stock 6 cm plates in the cold room. These are usually made every weekday, although there are times when demand exceeds the supply. Everyone makes his or her own 3.5 and 10 cm plates. Occasionally people may be able to spare a few, but if you know you are going to need a lot it is best to keep a personal stock in the cold room.

Plates can be poured in the cell culture room (Cook 3110) in the laminar flow hood closest to the sink. This hood can get quite crowded with stacks of plates, so please label your plates with name and date and remember to remove them as soon as they dry (usually the next day or two).

NOTE: 3.5 and 6 cm plates are stored under the table in the middle of the cell culture room while 10 cm plates are stored on top of the refrigerator in Cook 3103. Do not use the 10 cm plates on top of the refrigerator in the cell culture room as these are for cell culture.

Growing OP50 E. coli

1. Using sterile technique, inoculate Luria-Bertani (LB) media (stocks are located next to the main sink in Cook 3107) with a thawed vial of OP50 (located in the -70ºC stock freezer in Cook 3114).
2. Grow at 37ºC in the shaker (Cook 3114) overnight.
3. Use immediately or aliquot into 50mL vials for storage at 4ºC for a couple weeks.

NOTE: It is a good idea to make sure your OP50 is not contaminated by test seeding 5-10 plates and waiting a couple days to make sure nothing unwanted grows.

Seeding NGM Plates

1. Using sterile technique, pipette the desired amount of OP50 onto the dried NGM plates. The OP50 bacterial lawn should cover about 1/3 of the total plate surface area. As a general rule, you should use about 1 mL for 10 cm plates, 300 µL for 6 cm plates, and 200 µL for 3.5 cm plates. Try to keep the OP50 in the center of the plate (if the OP50 touches the side of the plate, there is greater risk of contamination).
2. Let the plates dry at room temperature. 6 and 3 cm plates can dry in about 24 hours and 10 cm plates take about 2 days to thoroughly dry. For stickier bacteria, let the plates dry for about 3 days at room temperature.
   NOTE: During humid or rainy weather the OP50 generally takes longer to dry.
3. Store plates in the cold room (4ºC) in a labeled box with a lid. They should be fine for several weeks.
Maintaining and Storing Worms
From Cold Spring Harbor Laboratories C. elegans Course 2007

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 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.

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 arrests growth of worms on the short term; 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.

Cryogenic Storage Any strain you value should be maintained in a permanent frozen collection. In the Morimoto lab common stock frozen strains are stored in the –70°C stock freezer (Cook 3114) and backups in the liquid nitrogen tank (Cook 3107, next to the worm incubators). For more information on frozen stock strains, see pages 12-13. See page 27 for freezing protocol.

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 distilled water and let soak for a few hours. Pour the excess water onto 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.

Morimoto lab addition:

NOTE: Any strains kept in the 15°C incubator or either of the 20°C incubators should be kept in a clean, labeled box with a lid (mite paper is optional). Boxes should be checked at least once a week for contamination.
Without a doubt you will have some form of contamination at some point. Although most contaminants do not harm the worms, it can make viewing the worms on the plate quite difficult.

**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 alkaline hypochlorite treatment. 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 pages 25-26 for the protocols. 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 the Morimoto lab, every couple of months there is generally a couple mite sightings. Thankfully these are usually isolated occurrences. However, there have been outbreaks in one or two of the incubators in the past. 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. Most people also use mite paper (located beneath the main sink in Cook 3107- the rolls with the hideous designs on them) in their boxes and on their benches. Eventually the paper does lose potency so it should be replaced every few weeks. Also, wear gloves or wash your hands after handling it.
Generating Males
From WormBook: The Online Review of C. elegans Biology. Genetic Mapping and Manipulations

Probably the best current way to generate males from a hermaphrodite stock is by placing hermaphrodites on RNAi feeding plates) that lead to a high incidence of males (Him phenotype). This occurs because loss of the Him gene activity leads to an increase in the spontaneous occurrence of haplo-X progeny. Many labs use an RNAi construct that targets him-14. RNAi feeding of him-14 will produce sufficient males within one or two generations to set up several plates for maintaining a long-term stock (if needed). One can also use actual Him mutant strains (such as him-5 or him-8), which produce 20-40% males at each generation. Depending on your intended use, however, it may not be convenient to have your constructed strains throwing large numbers of male self-progeny in future generations.

In addition to these approaches, males can also be generated following a mild heat shock of gravid adult hermaphrodites (34°C for 3-4 hours), although the frequency of males obtained using this method is relatively low (see page ____ for generating males via heat shock).

Maintaining Males
From WormBook: The Online Review of C. elegans Biology. Genetic Mapping and Manipulations

Once you've got your male stock, you will often want to keep it going indefinitely. Place about a dozen males on a plate with 3 or 4 hermaphrodites. Usually several plates are kept going, and the process is repeated every few days (at 20°C) or perhaps once a week (at 15°C). Here are a few hints for success, which also apply to all matings you may care to set up.

- Do not use old hermaphrodites! They are past their prime and will not work well. The best hermaphrodites to use are very young adults that have few or no eggs. It is better to set up matings using L4s than aging gravid adults.
- Males should also be on the young side (although this is somewhat less critical).
- Matings will usually work best if the bacterial spot is not too large and does not contact the edge of the plate.
- If you are in desperation, it is permissible to set up matings with animals that may be somewhat starved. Males seem to recover quite rapidly once placed on plates with food, and hermaphrodites also do reasonably well, provided they are picked as L4s or very young adults.

Should your homozygous male stock become contaminated, transfer several dozen males and hermaphrodites to a single plate, incubate overnight, and hypochlorite treat the hermaphrodites the next day. Alternatively, if you can find a mating plate where there are many males and gravid adults, simply hypochlorite treat the hermaphrodites (30-80, using several plates if necessary), and sufficient clean males and hermaphrodites should be recovered by the next generation.
Using the Morimoto Lab Strain Database

The strain database can be found on the computer in Cook 3104 and also on the Morimoto server.

Database Login Information
Account Name: morimoto
Password: stress

Every entry in the database represents one vial in either the -70°C freezer stocks (Cook 3114) or the backup liquid nitrogen (N2) stocks (Cook 3107). Each entry has four tabs for different types information: where the strain came from, a description of the strain, freezing information, and any associated publications.

To search the database, either go to View → Find Mode or click the magnifying glass symbol in the upper left hand corner. You can then search using any text field on any of the four tabs. However, most people search by either the ‘Strain Name’ or ‘Key Words’ located at the top of the page. See next page for an example of a vial entry page.

Removing a Strain from Morimoto Lab Common Stock

When you find a strain you would like to use, go to the ‘Freezing’ tab and see whether it is in -70°C freezer stocks or the liquid nitrogen tank. Note the box and slot number. For the liquid nitrogen tank, the ‘Box #’ is made up of the column the box is in and then the box number. For example, if the box number was 2.4, the box would be in the second column and the fourth box down.

Next, simply go to either the -70°C freezer or the liquid nitrogen tank and remove the strain you need. You must then sign out the strain you took on the clipboard located on the door of the -70°C freezer and on the side of the incubator next to the liquid nitrogen tank. This only takes a second to do, but it is very important. Every week or so, the clipboards are used to update the database. This way no one will go looking for a strain that has already been removed.

NOTE: Please remove vials from the -70°C freezer before going into the backup liquid nitrogen tank stocks. However, sometimes the only vials available are in liquid nitrogen.

NOTE: If there is only one vial left of a particular strain in the database, please make note of this on the sign-out clipboard and/or tell the person in charge of the database.
Vial is located in the -70°C freezer stocks in box 51, slot 13.
**Useful Websites**

From WormBook: The Online Review of *C. elegans* Biology. Web Resources for *C. elegans* Studies

**WormAtlas**

<http://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**

<http://www.wormbase.org>

WormBase is a major repository for *C. elegans* information, including genomic, genetic, anatomy, people, and literature. Access to information is via a set of Web pages, each of which 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 Web page of one type to another. For example, one 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 a link again to review an expression pattern description on an expression pattern page, click yet again to read about a cell on an anatomy page, and so on.

To look up worm strains, genes, alleles, etc. go to: <http://wormbase.org/db/gene/strain>.

**WormBook**

<http://www.wormbook.org>

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

**Caenorhabditis Genetics Center (CGC)**

<http://biosci.umn.edu/CGC>

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.

**Caenorhabditis elegans WWW Server**

<http://elegans.swmed.edu>

This site is a well-organized portal of many different types of information useful to *C. elegans* researchers. It is organized in two layers. The front page lists major topics or interests. Each topic has a hyperlink to a page that either offers a service (e.g. Literature Search) or is a list of links to other Web sites that offer services.
Useful Books
These books are located in Cook 3104 below the computer when you first walk in.


Morimoto Lab Server (Name: worm, Password: stress)
In the ‘Documents’ folder there is a folder entitled ‘CSH Worm Course 8/07’ that contains all the material from the Cold Spring Harbor Laboratory annual *C. elegans* course.
Caenorhabditis Genetics Center (CGC)
From WormBook: The Online Review of *C. elegans* Biology. Maintenance of *C. elegans*.

The CGC was established at the University of Missouri, Columbia, in 1979. In 1992 the CGC moved from Missouri to the University of Minnesota, St. Paul. Funded through a contract with the National Institutes of Health National Center for Research Resources (NIH NCRR), the basic mission of the CGC is to provide *C. elegans* strains and information to scientists initiating or continuing research using *C. elegans*. The CGC strives to acquire and have available for distribution stocks representing at least one mutant allele of each published gene and all chromosome rearrangements (deficiencies, duplications, translocations, inversions). In addition, many wild-type isolates of *C. elegans* and several species closely related to *C. elegans* are available. The CGC maintains an up-to-date bibliographic list of all articles, reviews and books that discuss *C. elegans*.

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 (From WormBook: The Online Review of *C. elegans* Biology. Maintenance of *C. elegans*).

To request a strain while in the Morimoto lab, please talk to lab manager or the current worm technician. There is usually about a two week wait time to receive a strain, so try to plan ahead.

NOTE: Recently the CGC has started charging $7 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 the worm technician so they can add it to the lab common stocks. Not only is this a way to cut down on multiple orders of the same strain, but it is much faster to simply take the strain from our freezer instead of waiting two weeks for it to be sent from the CGC.

Submitting a Strain to the CGC

Again, please talk to the lab manager or the lab’s worm technician. You should provide them with 1 or 2 plates and the following information: strain name, genotype (including gene names and alleles), name of person who made the strain, mutagen used (if any), number of times outcrossed, phenotype, culture conditions, and references.
Worm Nomenclature 101
By Cindy Voisine

Lab Identification
Strain code for naming strains
- Morimoto AM (Example: AM140)
- Sydney Brenner CB
- Bob Horvitz MT
- Cynthia Kenyon CF

Allele code for mutations/transgenes
- Morimoto rm (Example: rm7)
- Sydney Brenner e
- Bob Horvitz n
- Cynthia Kenyon mu

Gene Names
Rule:
- 3 or 4 letters, hyphen, number
- italicized
- lowercase
- *followed by linkage group (chromosome)

Examples: unc-54 I
let-858 II

* These statements are recommendations, not rules.

Alleles/Mutations
Rule:
- every mutation has a unique allele assignment
- italicized letter(s) followed by arabic number
- use gene name followed by allele in ()
- *mutations that are temperature sensitive can be designated as ‘ts’ following allele name
- *wildtype alleles have + sign in ()
- if deletion takes out more than one gene Df
- knockout consortium allele codes are ok, bc, and tm

Examples: unc-30(rm7) daf-16(mgDf50)
unc-15(e1402ts) dpy-5(+)

Transgenes
Rule: - Italicized name with allele \((rm)\) prefix, \(Ex\) and a number
- Integrated arrays are \(Is\)
- Mossci 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.

Example: \(rmIs18[dpy-5(+)unc-54p::GFP]\) I made up this strain.
\(vha-6::GFP\)
\(vha-6p::GFP\)

CRISPR

Rule:
- gene name for location of CRISPR insertion followed by () containing an allele name
- \(*\)Can be followed by brackets containing insertion information
- \(*\)Can be followed by chromosome number.

Example: \(lgg-1(rm17[lgg-1::mcherry])\) II.
This nomenclature denotes the endogenous lgg-1 locus tagged with mcherry.

Multiple Mutations in a Strain

Rule: - 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)

Examples: \(lin-12(n137)\) III; \(him-5(e147)\) V
\(dpy-5(e61)\) \(unc-13(+)dpy-5(+)\) \(unc-13\) (e51) I

Protein/RNA Names

Rule: For Protein, refer to gene name
- non-italicized and capitalized
- those with alternate splicing get a 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

Examples: \(UNC-54\) \(PQE-1(P79Q)\)
\(PQE-1A\) \(UNC-54\) mRNA levels
\(PQE-1B\) \(let-7\) mRNA
Phenotypes
Rule:  - non-italicized 3 or 4 letter abbreviation that usually corresponds to a gene name
       - first letter is capitalized
       - when referring to wildtype call it non-
       - RNAi phenotype use gene name with (RNAi)

Examples: Unc  non-Unc  mog-4(RNAi)
          Dpy      Daf
          Osm      Muv

Strain Names
Rule:  - nonitalicized names consisting of 2 or 3 capital letters followed by a number
       - every cross requires a new strain name
       - backcrossing does not (specify in database).
       - each independent extrachromosomal array line has a new strain name
       - each independent integrated array has a new strain name

Examples: AM565
          CF1057

‘Test’
- daf-16 mutant number 86 from Cynthia Kenyon’s lab. daf-16 is on chromosome I
- crossed to strain expressing a promoter fusion of C12C8.1 to GFP marked with a rol-6
  dominant mutant with allele number 1006 from Henry Epstein’s lab with an allele
  designation (su) integrated on chromosome II
- inject an extrachromosomal array using HSF-1 tagged with RFP under its own promoter

Generate 3 lines.

Our strain database last entry: AM702
Our last entry for arrays: rmIs47 and rmEx182

What is/are the strain name(s?) and genotype(s)?
Common Phenotypes
From WormBook: http://www.wormbook.org/chapters/www_introandbasics/introandbasics.html

Dumpy (Dpy): short and fat phenotype. Different dpy mutants can range from severe (small footballs) to moderate (slightly pudgy) in character. The more severe ones will often display a variable Unc phenotype as well.

Uncoordinated (Unc): There are many different subclasses of unc mutants. These include coiler Uncs, kinker Uncs, paralyzed Uncs, shrinker Uncs, Uncs that fail to move backwards when touched with a pick on their heads, Uncs that display poor forward movement but back well, etc. Recognizing certain types of Uncs can initially be challenging, although it usually gets easier over time. Often Uncs are somewhat misshapen and are typically smaller or thinner than wild-type animals.

Small (Sma): These animals tend to be more proportional in shape than Dpy animals; less stocky, more like wild type.

Long (Lon): These animals can often be a bit on the thin (stringy) side. Although Dpy and Sma animals can in some cases be very small as compared with wild type, even the longest Lon is only about 50% greater in length than wild-type animals.

Egg-laying defective (Egl): This can lead to the Bag (bag of worms) phenotype where embryos hatch within the mother, leaving a cuticle sack containing multiple wriggling larvae. Egl animals can be recognized before bagging as adults that seem to be bloated with eggs. Caution must be used, however, as aging wild-type hermaphrodites can often appear somewhat Egl over time. An individual bag will only last for about 24 hours (at 20° C) on the plate. Once worms become starved, the incidence of Egl's and Bags in genetically wild-type animals increases substantially. Unambiguous identification must always be carried out on non-starved plates.
Lethal (Let): These can range from embryonic lethals that never hatch to lethals that die as larvae. The latter category is easier to recognize, especially when the worms display a distinct larval lethal phenotype such as a "paralyzed rod" or a severe Dpy. Dead or dying eggs can be difficult to distinguish from healthy wild-type eggs on first viewing with a dissecting scope. To "see" embryonic lethals, one must allow a parent to lay eggs for a set period of time (usually 3-12 hours). The parent is removed to a new plate, and the fates of the eggs are followed. The presence of lethals can usually be identified unambiguously after about 18 hours (at 20°C) when the vast majority of wild-type embryos would have already hatched. Other designations for embryonic lethal mutations include emb and zyg.

Sterile (Ste): These animals come in several varieties. The most useful for mapping are those in which the sterility is obvious because the adult worms are devoid of eggs. Care must be taken to avoid mistaking a sterile animal for one that is merely a young adult that does not yet contain obvious eggs. If in doubt, transfer the suspected sterile animal to a new plate and follow its fate. In some cases, sterile animals may contain a protruding vulva (Pvl-sterile), which makes identification facile. So called "maternal-effect" lethal mutants are really just sterile animals that contain dead eggs and are therefore harder to recognize.

Roller (Rol): Animals form a horseshoe shape and tragically twist in place about their long axis. The Rol phenotype can be masked by strong unc or dpy mutations, which prevent the animals from carrying out the classic roller moves.

Blister (Bli): Adult animals have a variably blistered cuticle, which can resemble a large bubble on the surface of the worm. The Bli phenotype can be suppressed by a number of dpy and rol mutations.

Lineage defective (Lin): These can display any number of distinct defects in the pattern of cell divisions that occur during development. Consult WormBase or other sources for specifics concerning the defects associated with particular mutants.
Microscope Usage

Cook 3107A Microscopes (‘Worm Room’)
The worm room has five microscopes, three small dissecting microscopes (Leica MZ12.5, Leica MZ6, Leica MZL2) and two larger microscopes with fluorescent capabilities (Leica MZFLIII and Leica MZ16FA). You do not need to be trained on the dissecting microscopes since they are fairly straightforward to use. However, you must be trained on the fluorescent microscopes before you are allowed to use them. Please see Tali Gidalevitz to set up a training time. It shouldn’t take more than 5 to 10 minutes.

Cook 3112 Microscopes (‘Injection Room’)
There are three large microscopes in this room. The Leica DM IRB is for injection purposes only and should not be used unless you are trained on microinjection. The other two microscopes have both fluorescent and digital picture-taking capabilities. Again, you must be trained on these microscopes before using them. As with the worm room fluorescent microscopes you need to see Tali Gidalevitz for training on the Leica MZFLIII. See Daniel Czyz for training on the Axiovert 200.

General Microscope Policies

- There are sign-out sheets next to each microscope, both in the worm and injection rooms. Please indicate how long you will be using the microscope so that others can plan their day too.
- If you do not use the full amount of time you signed out for or if you realize you do not need the time you are signed up for, please cross your name out to let other lab members know the microscope is available to give other people a chance to use it.
- If you smudge the eyepieces or a lens, please clean it off with lens paper (NOT a paper towel or a Kim wipe).
- For the fluorescent microscopes: if no one is signed up to use it after you, please turn the microscope off. However, once a fluorescent bulb has been turned on, you need to wait about 15 minutes before turning it off again.
- For the dissecting microscopes: please turn them off as soon as you are finished with them. These can be turned off and on without waiting for it to cool down.
- In the evenings (around 5:00pm) all the microscopes should be covered to protect them from dust. If the fluorescent microscopes were recently turned off, the fluorescent power box will be quite warm to the touch. Leave this part uncovered to prevent the cover from getting to too hot.
- If someone needs a microscope for most or all of the day (such as when someone is doing a genome-wide screen) they should email the lab a week before to notify people that particular microscope will be unavailable on the mentioned day.