Experiment 1 Gene Transfer and Maintain

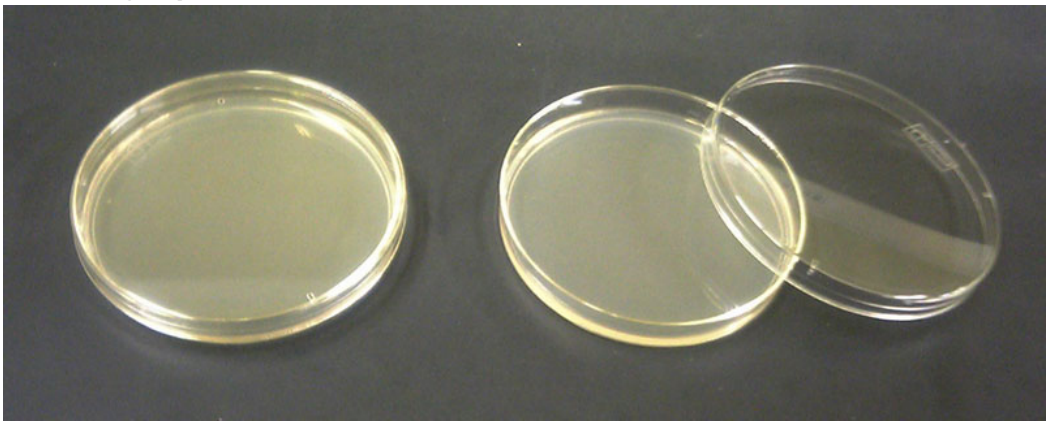
C. elegans is a commonly used model system, though it is not popular in Taiwan, it is widely used in foreign countries. It even plays an important role in many Nobel Prize winning research.

In this course, you will learn: 1. How to pick and transfer *C. elegans* and do “chunking“ 2. How to preserve *C. elegans* strains 3. Generate males by heat shock 4. How can one tell the differences between different stages of worms and the difference between male and hermaphrodite 5. Observe primary neuronal cells isolated from the worm.

Maintaining and growth of worms

C. elegans is maintained on nematode growth medium (NGM) agar which has been aseptically poured into petri plates. Drop *E. coli* OP50 (an uracil auxotroph *E. coli* strain served as worms’ food) liquid culture to the NGM plates using a pipet. Allow the *E. coli* OP50 lawn to grow overnight at room temperature. When dropping, take care to keep the lawn in the center and the worms will tend to spend most of the time in the bacteria. All the strains in this project are maintained at 15-23°C. When OP50 is worn out, use a sterilized scalpel to move a “chunk” of agar (containing several worms) from an old plate to a fresh plate or pick single worms by platinum wire picker.

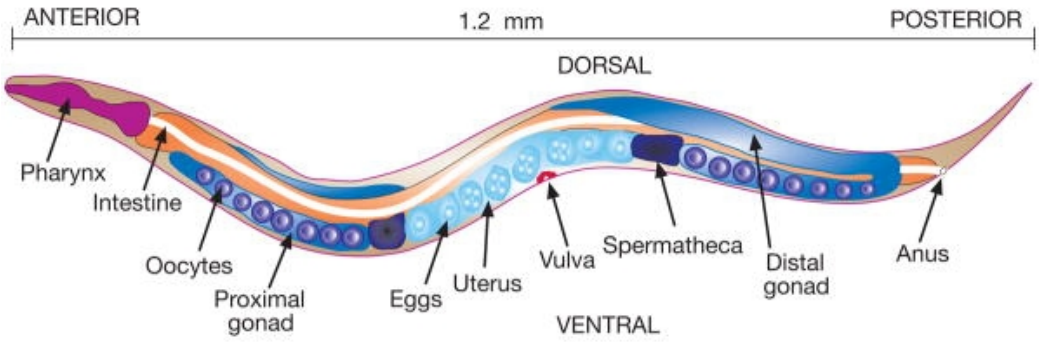
NGM agar plates

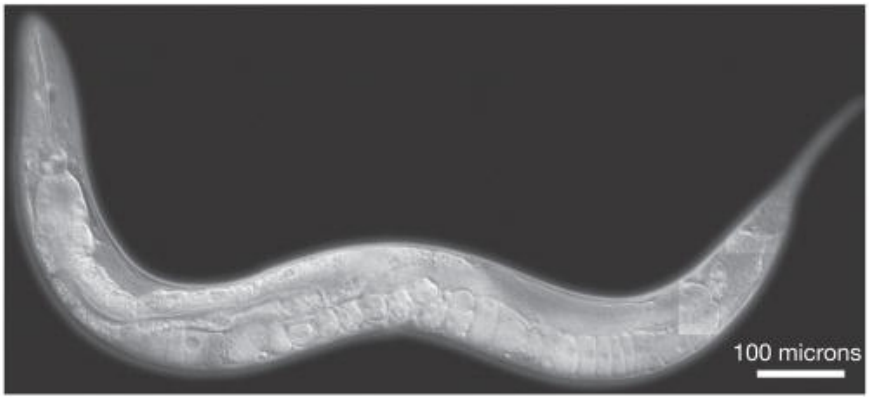


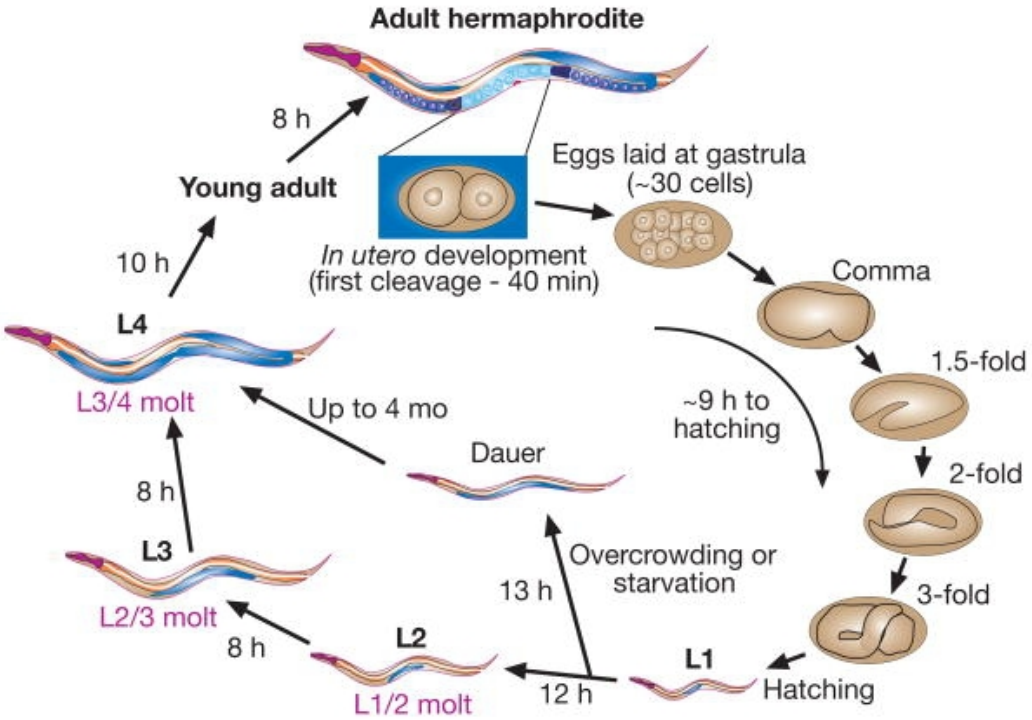
Freezing and recovery of *C. elegans* stocks

Caenorhabditis elegans can be frozen and stored indefinitely in liquid nitrogen (−196 °C) (Brenner, 1974). The keys to a successful freeze are using animals at the correct stage of development, the addition of glycerol to the freezing media, and a gradual cooling to -80°C. Freshly starved young larvae (L1-L2 stage) survive freezing best. Well-fed animals, adults, eggs and “Dauers” (a stress and food-resistant form) do not survive well. It is best to use several plates of worms that have just exhausted the *E. coli* OP50 lawn and that contain lots of L1-L2 animals. A 15% final volume of glycerol in the freezing solution is used. A 1°C decrease in temperature per minute is desirable during freezing. This can be achieved by placing the worms (in freezer vials) in a Nalgene Cryo freezing container at -80°C, with 100% isopropanol that can repeatable -1°C/minute cooling rate. After 3 or more hours at -80°C, the freezer vials should be transferred to their permanent freezer location for long term storage.

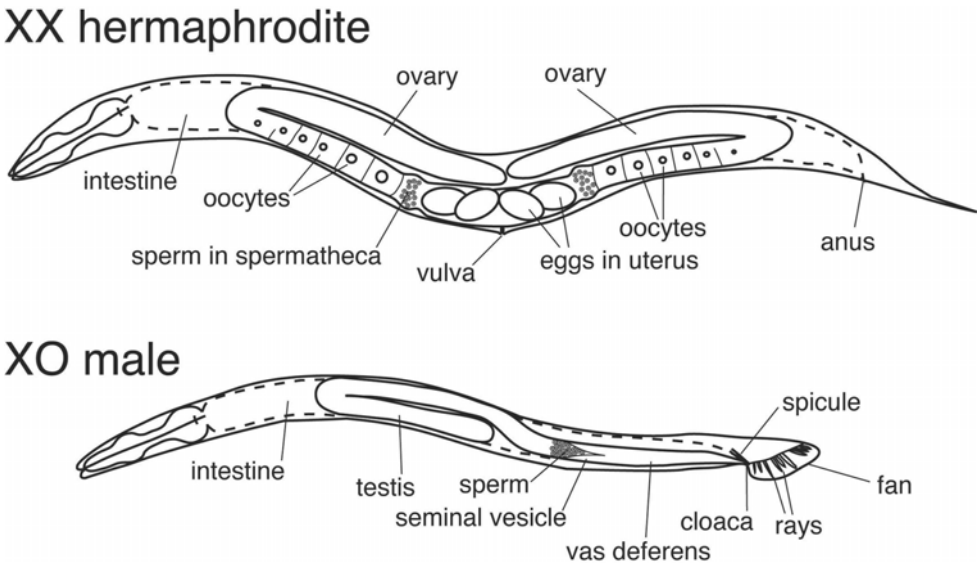
Different stage and the structure of C.elegans







Different sex of C.elegans



Homework

1. Please write down the differences between male and hermaphrodite worms.
2. Please draw a *C. elegans*' neural cell. Does it have a myelin sheath?
3. Please calculate the percentage of survival after thaw.
4. After heat shock experiment, please calculate the amount of gained male worms. Note down how many males you have got.

Procedures and Reagents

Freezing *C. elegans* and Recovery

Strain: Punc-104::UNC-104::GFP(e1265)

Reagents: M9 Buffer, Freezing Buffer

Freezing

1. Wash the plates with M9 Buffer, collect liquid in a 15ml centrifuge tube. Centrifuge for 3 minutes 1,300rpm at 22°C.
2. Wash worms for another 2 times. (M9 Buffer, 1,300rpm, 3 minutes, 22°C)
3. Remain 1ml pellet of worms, and then add 1ml Freezing Buffer into the tube. Mix well.
4. Transfer 0.9ml mixture into the labeled cryotube (Total: 2 cryotubes).

5. Put the cryovials in a Nalgene freezing container.
6. Put the Nalgene freezing container in a −80°C freezer for at least 3 hours.
7. The next day transfer the vials to their permanent freezer locations.

Recovery

1. Remove a vial from the −80°C freezer.
2. Defrost the vial as quick as possible.
3. In the flow, transfer the solution to a NGM plate with OP50 lawn.
4. Incubate at 22°C for recovery.

Generating males by heat shock

Strain: Punc-104::UNC-104::GFP(e1265)

1. Pick 5 L4 hemaphordites in a small NGM plate with OP50 lawn. Prepare 6 plates.
2. Incubate 5, 5.5, and 6 hours at 30°C. Incubate 2 plates for each time-length.
3. Return to 22°C. Find males from the F1 generation.

Maintaining worms

Strain: N2

Chunking

1. Use scalpel to cut out a cube of plate.
2. Transfer the cube up-side down on a new NGM plate with OP50 lawn.

Picking

1. Use flattened tip of a platinum wire to pick up worms and transfer to a new NGM plate with OP50 lawn.

Please find the "Methods" from below connection to learn Chunk and Pick.
http://www.silencinggenomes.org/animations/Lab2_Culturing_Celegans.swf

Reagents

NGM agar plates (Nematode growth medium agar paltes)

Dissolve 3g NaCl, 2.5g Peptone and 20g Agar in 1 liter of Milli-Q water and autoclave. After cooling to 55℃, under sterile conditions add the following solutions in the given order, while stirring: 1 ml 5 mg/ml cholesterol in ethanol, 1 ml 1M $CaCl_2$, 1 ml 1M $MgSO_4$ and 25 ml 1M KH_2PO_4 (pH 6.0). Pour liquid agar medium into Petri dishes.

M9 Buffer

Dissolve 3g KH_2PO_4, 6g Na_2HPO_4, 5g NaCl, in 1 liter of Milli-Q water and autoclave. After cooling to 55℃, under sterile conditions add 1 ml 1M $MgSO_4$.

Freezing Buffer

Mix following solutions: 20 ml 1M NaCl, 10 ml 1M KH_2PO_4 (pH 6.0), and 60 ml (min. 99%) glycerol. Add Milli-Q water to 200 ml and autoclave. After cooling to 55℃, under sterile conditions add 0.6 ml 0.1M $MgSO_4$.