

Synchronise by bleach

1. Use dry plates to allow quick absorption of the bleach drop.
2. Dilute hard core bleach solution 1:1 with distilled water.
3. Collect adults in side of plate without food and drop 10 μ l diluted bleach solution on them.
4. The next day⁽¹⁾ chunk the emerged worms that have moved to food into a new plate.

L1 preparation

Materials needed

- Bleach (NaOCl black bottle, keep at 4°C)
- M9
- 10 M NaOH
- Milliq water (or distilled water from tap)
- Always work close to flame

Protocol

1. Make **bleach mix**: For 8 tubes (3 ml each): 5 ml bleach, 1 ml **10M NaOH**, 19 ml **H₂O**.
2. Wash worm plate with 2 ml **M9**.
3. Transfer worms to a 15 ml tube, add to 15 ml **H₂O**.
4. Centrifuge at 1000 g 3 min, remove supernatant.
5. Add 2-3 ml **bleach mix**.
6. Incubate 3-6 min at RT (shake a few times), until 70% of worms are broken and eggs are released⁽²⁾.
7. Immediately⁽³⁾ add to 15 ml **M9**.
8. Centrifuge at 1000 g for 3 min.
9. Remove supernatant (with pipette only), resuspend in 10 ml **M9**.
10. Centrifuge at 1000 g for 3 min.
11. Remove supernatant, resuspend in 1 ml **M9**, and transfer to small sterile dish.

Worm transfer to assay plate

1. Pipette 2 ml **M9** on a plate with adults and transfer to eppenforf.
2. Centrifuge 1 min at 3,000 RPM.
3. Remove supernatant, add **M9**, check it is empty of bacteria. Repeat wash if needed.
4. Resuspend worms in a small volume and transfer to assay plate with glass pipette⁽⁴⁾.

Male generation

1. Set up ~6 plates with 5 L4 hermaphrodites each.
2. Heat shock 4-6 hours at 30°C.
3. Return to 20°C. Should get a few males per plate in the F₁.
4. When L4 appear, transfer mothers to new plate. Usually the males are not the first to appear so if there is

no male on day 4 keep looking at the plate the next 2 days.

5. Cross the males with L4 hermaphrodites to get 50% males in the next generation.
6. Maintain male stocks by crossing 12 males with 3 L4 hermaphrodites in each generation.

Freezing protocol

Materials needed

- M9 buffer
- Freezing buffer
- -80°C space
- 2 polystyrene racks from 15 ml Falcons

Protocol

1. Grow and starve⁽⁵⁾ 2-3 plates⁽⁶⁾.
2. Arrange 3 Nunc cryotubes with color caps and label with strain name on the cap. Also add in Excel.
3. Wash each plate with 1000 μ l **M9 buffer** and transfer to 2 cryotubes.
4. Add 350⁽⁷⁾ μ l **freezing solution** to each cryotube and shake briefly to mix.
5. Place tubes in a polystyrene rack⁽⁸⁾ and overlay a second, inverted rack on top.
6. Store at -80°C overnight (or at least 12 hours) and transfer the tubes to their permanent locations the following day.
7. Fill-in the Excel file.
8. 1-2 tubes will remain in the -80°C freezer as a working stock. 1 tube should be moved to liquid nitrogen storage to serve as backup.
9. Thaw a tube after freezing to check it for survival. Pour most of tube in one plate (backup) and remaining drop to other plate. Pick animals that move to food into new plate. This removes contaminants and avoids overcrowding.

DNA extraction

Materials needed

- 10 mg/ml Proteinase K
- 10X PCR buffer

Protocol

1. Prepare pcr tubes with 20 μ l **1X PCR buffer**.
2. Add 1 μ l **proteinase K**.
3. Add 10 adults or 20 L4 worms to each pcr tube.
4. Freeze at -80°C for 20 min to break up worms.
5. Put in WORM_DNA⁽⁹⁾ program
 - 65°C for 1 hr
 - 95°C for 10 min

⁽⁵⁾ Freeze the worms within one day of starvation, if possible. Freshly starved L1s and L2s are best for freezing.

⁽⁶⁾ The plates should be clean although fungal contamination has never been a problem.

⁽⁷⁾ The final mix is 1:1 because you will not recover all M9 from the plate.

⁽⁸⁾ The one from 15 ml falcons works well.

⁽⁹⁾ Machine 2.

⁽¹⁾ Worms are stressed the longer they stay on the plate with bleach, even though most sinks to the bottom of the plate.

⁽²⁾ Check under dissecting microscope.

⁽³⁾ Bleach is toxic to eggs.

⁽⁴⁾ Worms stick to plastic.

- Keep at -20°C if not use immediately. $2\ \mu\text{l}$ in $20\ \mu\text{l}$ PCR volume.

Recipes

Egg buffer (500 ml)

- Assemble the following components at room temperature and bring to 500 ml with ddH₂O.

12.5 ml	1 M HEPES in ddH ₂ O, pH 7.3	4°C
29.5 ml	2 M NaCl in ddH ₂ O	RT
12 ml	2 M KCl in ddH ₂ O	RT
1 ml	1 M CaCl ₂ in ddH ₂ O	RT
1 ml	1 M MgCl ₂ in ddH ₂ O	RT

- Adjust osmolarity to 340 ± 5 mOsm by diluting with sterile ddH₂O.
- In a tissue culture hood, sterilize the buffer with a $0.22\ \mu\text{m}$ vacuum filter unit.
- Store at 4°C .

Freezing solution (1l)

- Dissolve the following in 700 ml distilled H₂O.
 - 0.78 g K₂HPO₄
 - 8.28 g KH₂PO₄
 - 4.1 g NaCl
- Add 300 ml glycerol while stirring.
- Aliquot and autoclave.

M9 (Minimal Media) (1x)

- Dissolve the following
 - 5.8 g Na₂HPO₄ (Anhydrous)
 - 3 g KH₂PO₄ (Anhydrous)
 - 0.5 g NaCl
 - 1 g NH₄Cl
- Make final volume to 1 L.
- Autoclave.

Hard core bleach

- Dissolve the following
 - 0.25 ml 10M NaOH
 - 1 ml of NaClO (6-14% active chlorine (Merck))

NGM plates (1 l)

- Mix
 - 17 g Agar
 - 3 g NaCl
 - 2.5 g Peptone
 - 1 ml Cholesterol (5ml/ml in ethanol)
- Make final volume to 973 ml. Autoclave, then add separately:
 - 1 ml 1M CaCl₂
 - 1 ml 1M MgSO₄
 - 25 ml 1M K₂HPO₄ pH 6.0
- Phosphate solution (Sterile) added after cooled to below 60°C
 - 0.17 M KH₂PO₄ + 0.72 M K₂HPO₄

Autoclave

- Cover bottom with water
- Turn on green switch
- Press black button until the screen turns on

- On screen press
 - set -> 120 (temp)
 - set -> 120 (temp)
 - set -> 60 (min)
 - hold set 5 sec to start (c1 appears)
- While running
 - Make sure top valve is loose and down
 - Check at 30 min all ok
- Can release valve at 5-10 pressure

NGM plates (400 ml)

- In a 500 ml bottle add
 - 8 g Agar
 - 1.2 g NaCl (N) or 2.4 g NaCl (H) or 0.9 g NaCl (L)
 - 1 g peptone
- Leave by autoclave.
- Autoclave
 - Add 400 ml distilled H₂O.
 - Autoclave 1 h
 - Leave at 55°C
- Pour to plates after adding
 - 400 μl cholesterol (from -20°C)
 - 400 μl MgSO₄
 - 400 μl CaCl₃
 - 10 ml KPO₄
- Add Bacteria from fridge
 - 50 μl for small plates, tilt to spread the solution and store upside down.
 - 200 μl for large plates, spread with glass spreader by flame.
- Leave at room temperature for 24 h, then store at 4°C room.

2xYT medium (100 ml)

- Measure 90 ml of distilled H₂O. Add
 - 1.6 g Bacto Tryptone
 - 1.0 g Bacto Yeast Extract
 - 0.5 g NaCl
- Adjust pH to 7.0 with 5N NaOH.
- Adjust to 100 ml with distilled H₂O.
- Autoclave.

PCR programs

WORM_ID

95°C	2 min	x35
95°C	30 s	
60°C	30 s	
72°C	1 min	
72°C	10 min	
12°C	forever	

10x buffer	2
CEL_F	1
CEL_R	1
CRE_F	1
CRE_R	1
dNTP	0.4
H ₂ O	11.4
Taq	0.2
DNA	2

C. elegans Life Cycle Stages

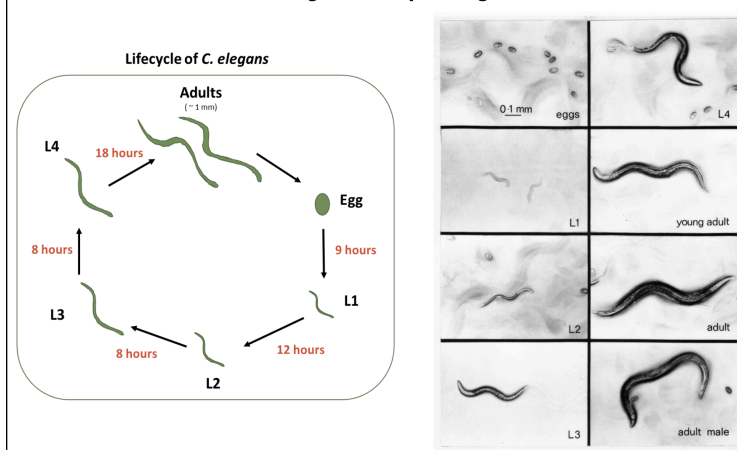


Figure 1: Life cycle diagram and pictures of *C. elegans*.

Table 1: Information on development time, body length and egg production at different maintenance temperatures.

	16°C	20°C	25°C
Egg laid	0 hr	0 hr	0 hr
Hatching	16-18 hr	10-12 hr	8-9 hr
1 st molt lethargus	36.5 hr	26 hr	18 hr
2 nd molt lethargus	48 hr	34.5 hr	25.5 hr
3 rd molt lethargus	60 hr	43.5 hr	31 hr
4 th molt lethargus	75 hr	56 hr	39 hr
Egg laying begins	90 hr	65 hr	47 hr
Egg laying maximal	140 hr	96 hr	62 hr
Egg laying ends	180 hr	128 hr	88 hr
Length at 1 st molt	360 µm	370 µm	380 µm
Length at 2 nd molt	490 µm	480 µm	510 µm
Length at 3 rd molt	650 µm	640 µm	620 µm
Length at 4 th molt	900 µm	850 µm	940 µm
Length at egg-laying onset	1150 µm	1060 µm	1110 µm
Maximal egg laying rate	5.4 /hr	9.1 /hr	8.1 /hr
Total eggs laid	275	280	170