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 µl 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

- 5 ml of bleach from the black bottle in the fridge
- 5 ml of NaOH 2M
- 15 ml of miliq water

Protocol

1. Wash worm plate with 2 ml M9.
2. Transfer worms to a 15 ml tube, add to 15ml ddH_2O.
3. Centrifuge at 500 g 3 min, remove supernatant.
4. Add 2-3 ml bleach.
5. Incubate 3-6 min at RT (shake a few times), untill 70% of worms are broken and eggs are released[2].
6. Immediately[3] add 15 ml egg buffer.
7. Centrifuge at 500 g for 3 min
8. Remove supernatent, resuspend in 10 ml Egg buffer.
9. Centrifuge at 500 g for 3 min
10. Remove supernatent, resuspend in 1 ml M9 or egg buffer, 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 asay 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 F_1.
4. When L4 appear, tranfer 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
- -80ºC space
- 2 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 µl **M9 buffer** and transfer to 2 cryotubes.
4. Add 350[7] µl **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 -80ºC 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 -80ºC 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.

Recipes

Egg buffer (500 ml)

1. Assemble the following components at room temperature and bring to 500 ml with ddH_2O.

12.5 ml	1 M HEPES in ddH_2O, pH 7.3	4ºC
29.5 ml	2 M NaCl in ddH_2O	RT
12 ml	2 M KCl in ddH_2O	RT
1 ml	1 M $CaCl_2$ in ddH_2O	RT
1 ml	1 M $MgCl_2$ in ddH_2O	RT

2. Adjust osmolarity to 340±5 mOsm by diluting with sterile ddH_2O.
3. In a tissue culture hood, sterilize the buffer with a 0.22 µm vacuum filter unit.
4. Store at 4°C.

Freezing solution (1l)

1. Dissolve the following in 700 ml distilled H_2O.
   - 0.78 g K_2HPO_4

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[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.

[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.

- 8.28 g KH_2PO_4
- 4.1 g NaCl

2. Add 300 ml glycerol while stirring.
3. Aliquot and autoclave.

M9 (Minimal Media) (1x)

1. Dissolve the following
   - 5.8 g Na_2HPO_4 (Anhydrous)
   - 3 g KH_2PO_4 (Anhydrous)
   - 0.5 g NaCl
   - 1 g NH_4Cl
2. Make final volume to 1 L.
3. Autoclave.

Hard core bleach

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

NGM plates (1 l)

1. Mix
   - 17 g Agar
   - 3 g NaCl
   - 2.5 g Peptone
   - 1 ml Cholesterol (5ml/ml in ethanol)
2. Make final volume to 973 ml. Autoclave, then add separately:
   - 1 ml 1M $CaCl_2$
   - 1 ml 1M $MgSO_4$
   - 25 ml 1M K_2HPO_4 pH 6.0
3. Phosphate solution (Sterile) added after cooled to below 60ºC
   - 0.17 M KH_2PO_4 + 0.72 M K_2HPO_4

2xYT medium (100 ml)

1. Measure 90 ml of distilled H_2O. Add
   - 1.6 g Bacto Tryptone
   - 1.0 g Bacto Yeast Extract
   - 0.5 g NaCl
2. Adjust pH to 7.0 with 5N NaOH.
3. Adjust to 100 ml with distilled H_2O.
4. Autoclave.

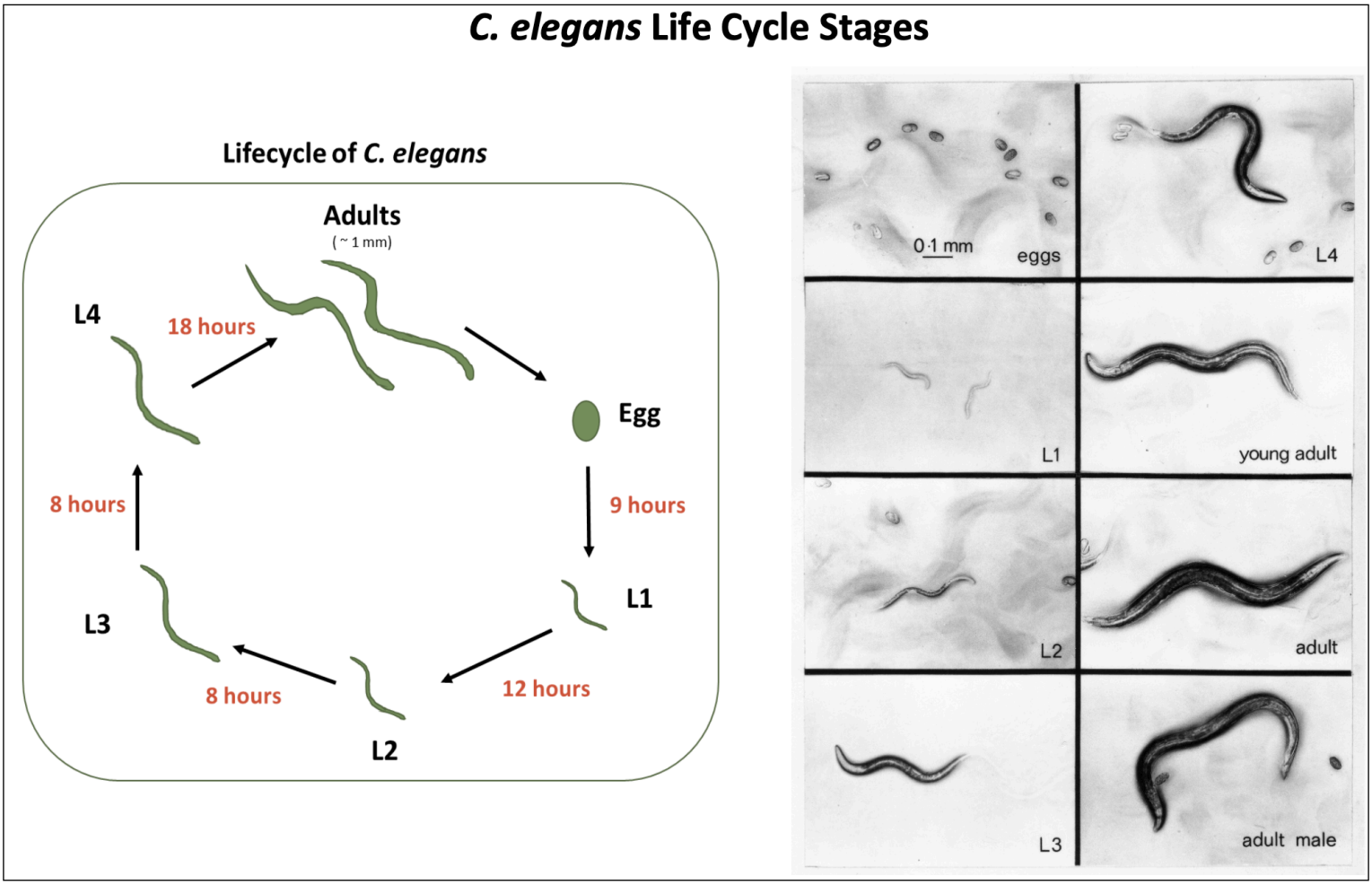

Figure 1: Life cycle diagram and pictures of *C. elegans*.

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} mool	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