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Measure 40 worm feeding with bioluminescent bacteria

Serena Ding[1]

[1]Imperial College London

1 Works for me dx.doi.org/10.17504/protocols.io.5hsg36e

Serena Ding

ABSTRACT

Protocol uses bioluminescently labelled bacteria (Gregor et al, 2008, Addgene Plasmid #107879) to measure population feeding of 40 adult C. elegans worms.

EXTERNAL LINK

https://www.pnas.org/content/115/5/962

MATERIALS TEXT

bioluminescence bacteria: Addgene Plasmid #107879
IVIS Spectrum imaging system (Caliper LifeSciences)
Living Image software (v 4.3.1)

ampicillin sodium salt (Sigma A9518)
LB broth and agar
NGM and low peptone NGM agar
M9 buffer
laminar flow hood (Heraguard)
spectrophotometer (Jenway7315)
centrifuge (Hettich Universal 320)
vortex mixer (Vortex-Genie 2, Scientific Industries)
styrofoam box
glass pipette
manual click counter

Bleach synchronise worms (Day -7 to -4)

1 Bleach synchronise animals from three 60mm plates, making sure lots of gravid hermaphrodites are present. Leave on rotator at 20 °C until use.

Bleach synchronisation of C. elegans PREVIEW RUN
by Ida Barlow

1.1 Wash hermaphrodites off plate with several ml of M9 solution and transfer to 15ml falcon tube (Fisher Scientific-Falcon 352096)

1.2 Fill falcon tube up to 15ml with M9 solution

1.3 Centrifuge for 2 minutes at 1500 rpm (RCF:210, ascending 9; descending 7) – program 1

Program 1 retains the worms as pellets and the bacteria is suspended as the supernatant

The descending is slow as the worm pellet is lose at this stage which we don't want to break

1.4 Remove supernatant using a plastic Pasteur pipette taking care not to disturb pellet
Leave atleast 0.5ml M9 to avoid disturbing the pellet

1.5 Fill the tube with M9 upto 15ml

1.6 Spin program 1

1.7 *Repeat steps 4-6*

1.8 On final wash remove as much supernatant as possible and add M9 upto 4ml

1.9 Add 4ml 2X Bleach solution (From here onwards try to work as quickly as possible to avoid over-exposure of the worms to the bleach)

USE FRESHLY PREPARED BLEACH EVERYTIME

2X Bleach solution:

5% Sodium hypochlorite solution - 4ml
Sterile water - 3.5 ml
1M NaOH solution - 2.5 ml
TOTAL - 10 ml

1.10 Vortex on maximum setting for 4 min (no more as this will damage the eggs)

Makesure the vortex forms

After vortexing, top up the tube with M9 till 15ml

1.11 Centrifuge for 2 mins at 2500rpm (RCF:590, ascending 9; descending 7) – program 2

(Always check the program on the centrifuge before using it)

1.12 Remove supernatant by pouring into waste bottle – pellet should be compact and yellow in colour at bottom of falcon, but be careful not to lose

1.13 Add 15ml M9

1.14 Centrifuge at program 2

1.15 Repeat steps 12-14 four more times

The number of washes is crucial here as we need to get rid of all the bleach

1.16 After final wash add 15ml M9 and store eggs/larvae in the falcon on the rotator that is constantly spinning at 20^{o}C, until feeding

L1 arrested larvae can be starved for up to 5 days before refeeding

1.17 Centrifuge larvae on program 2 to pellet

1.18 Remove supernatant with plastic Pasteur pipette

The pellet is lose here so makesure not to disturb it

1.19 Add 15ml M9, spin to wash

1.20 On final wash leave 0.5ml M9 in falcon

1.21 Resuspend the pellet by gently tapping the tube/flicking it

1.22 Place droplet containing larvae onto seeded plate and allow to grow to desired developmental state (ie. 2 days for L4s, 2.5 days for young adults)

Use glass pipette to place the droplet onto seeded plate, avoid using plastic pipette as larvae will stick to it

Development times at 20°C:

- 2 days for L4s
- 2.5 days for young adults

Note:

- If you feed larvae within 12hrs of bleaching then they develop faster than the longer arrested ones
- It is a good practice to bleach in two tubes in parallel
- If you drop the tube at any point of the process, makesure to transfer the contents into a new tube as the dropped tube may get cracked resulting in loss of worms during centrifugation/vortexing
- Any unused larvae can be topped up with M9 and stored spinning in the rotator to be re-used
- Use clean autoclaved rubber bulbs for the refeeding everytime to avoid contamination
- Put the used bulb in the box labelled 'Used Teets'

Stages	Grown at 20 C from L1	Grown at 25 C from L1
L1 division	11.7hrs	9hrs
Mid L1	16.9hrs	13hrs
First L2 division	22.1hrs	17hrs
Between L2 divisions	23.4hrs	18hrs
Second L2 divisions	24.3hrs	19hrs
Mid L2	29.9hrs	23hrs
L3 division	32.5hrs	25hrs
Mid L3	37.7hrs	29hrs
L4 division	42.9hrs	33hrs
Mid L4	49.4hrs	38hrs
Early adult	55.9hrs	43hrs
Adult	62.4hrs	48hrs

Table of Development times for different temperatures

Re-feed worms (Day -3)

2 Re-feed worms onto 60mm plates using a glass pipette. Incubate at 20 °C.

Monitor these plates until use to ensure worms do not run out of food.

Grow bacteria overnight

3 Day -2
Streak out bioluminescent bacteria frozen stock onto an LB plate containing 50 µg/mL ampicillin and incubate overnight at **37 °C**.

This step does not need to be performed every time. A streaked plate can be re-used within 1 month, so long as single colonies are still available. Otherwise re-streak onto a new LB/ampicillin plate for single colonies.

4 Day -1 ~5pm
To a 300 mL volumetric flask, add 100 mL LB broth, 100 µL ampicillin stock solution (50mg/mL) , and a single colony of bioluminescent bacteria from the streaked plate. Place on **37 °C** incubator shaking at 220 rpm overnight.

5 Day 0 ~10am
Take the bacteria overnight culture off of the shaking incubator and leave at room temperature on the bench until use that afternoon.

Do not put bacteria culture at 4 °C to save for later, as this will abolish the signal. Instead, a fresh overnight culture should be grown for each day of experiment.

Initialise the IVIS imaging system (Day 0 ~4.30pm)

6 Initialise the IVIS system via the LivingImage software.

7 Set up imaging parameters within the LivingImage software interface. Set stage temperature to **20 °C** , designate auto-saving directory for acquired images, and set up acquisition sequence parameters.

Bioluminescence acquisition parameters: subject height: 0.5 cm; field of view: C; expososure: 1 second; frame interval: 6 minutes; excitation: block; emission: open; luminescence binning: 4; photograph binning: 4; luminescence F/stop: 1; photograph F/stop: 1; batch sequence: true; sequence 1 length: 99; sequence 2 length: 35.

These imaging parameters have been determined in advance for the experiment and are saved as a .xsq configuration file, which can be loaded into the imaging software.

Seed bacteria for imaging (Day 0 ~4.30pm)

8 Take imaging plates (35 mm Petri dish containing 3.5 mL low peptone agar) out of the **4 °C** cold room, let them incubate at **Room temperature** for 30 minutes to warm up.

9 Give the flask containing the bacteria overnight culture a swirl to resuspend the bacteria cells that may have settled during the day.

10 Seed 20 µL of the bacteria culture onto the centre of each 35 mm imaging plate. Dry the plate in a laminar flow hood (setting II) for 30 minutes.

11 Measure bacteria culture optical density with a spectrophotometer for the record.

Inoculate worms for experiment (Day 0 ~5.30pm)

12 Retrieve imaging plates from the flow hood and label them with sample name.

13 Harvest synchronised adult worms using 2 mL of M9 buffer and transfer them into a 15 mL tube. Wash them twice with M9 buffer by topping up the tube with M9, spinning at 1500 rpm/210 rcf (ascending 9, descending 7), and then aspiring the supernatent leaving the loose pellet intact. After the last wash, leave the loose worm pellet.

This spinning programme separates worms (pellet) from bacteria (supernatant), so bacteria can be removed. Soft brake prevents the loose worm pellet from breaking up.

14 Using a glass pipette with a medicine dropper bulb attached, transfer 40 worms to each imaging plate. Use a manual click counter to count 40 worms.

Try to keep the droplet small so it gets absorbed into the media in a reasonable amount of time (a couple of minutes), although this makes accurate counting more difficult so it's worth practicing worm transfer beforehand to find the optimal worm transfer techniques.

It's handy to have a P20 pipette in hand with a few microliters of M9 already inside. To remove excess worms, lower the tip near the worms and carefully suck worms back out. It's not usually precise as to how many worms are removed, so repeat counting with a manual counter again after removing worms.

15 Place the lid back onto the imaging plate. When all worms are transferred, put all the samples inside a cardboard box and then inside a styrofoam box, and take them to the IVIS imaging system.

Carry carefully, as some liquid in which worms are transferred may not have tried completely and may disturb the bacterial lawn if it spills over.

Image (Day 0)

16 Make sure the liquid drop has been completely absorbed into the media. Vortex imaging samples for 10s on the lowest setting of a vortex mixer to disperse any existing aggregates in order to randomise the initial position of worms.

17 Place imaging samples inside the IVIS chamber inside the laser grid guidelines. Close the chamber door. Start acquisition via the software exactly 1 min after the vortex start.

Signal extraction and export (Day 1)

18 After image sequence acquisition, read signal across the acquisition sequence using the LivingImage software by measuring radiance (photons/s) for a defined region of interest.

19 Export signal as a tab-delimited text file for downstream analysis.