Feb 22, 2022

Keio Acute Response Antioxidant Rescue

Saul Moore[1]

[1]Imperial College London

dx.doi.org/10.17504/protocols.io.b3nnqmde

Behavioural Genomics

Saul Moore

Phenotyping the acute behavioural response of Caenorhabditis elegans (N2 Bristol) to *E. coli* single-gene deletion mutants (BW25113), in both the presence and absence of antioxidants (Trolox, NAC, vitamin C and resveratrol).

Videos are recorded at 25 fps on the laboratory's (Hydra) imaging rig, immediately after worms are picked onto imaging plates, for a total of 45 minutes at 25 fps, with blue-light stimulus delivered for 10 seconds in 5 minute intervals throughout the recording.

DOI

dx.doi.org/10.17504/protocols.io.b3nnqmde

Saul Moore 2022. Keio Acute Response Antioxidant Rescue . **protocols.io** https://dx.doi.org/10.17504/protocols.io.b3nnqmde

protocol ,

Jan 10, 2022

Feb 22, 2022

56750

Read through the whole protocol before starting, as the sections are not in chronological order

10 x 6-well plates (imaging plates)
10 x 60mm Petri plates (maintenance plates
3 x 90mm Petri plates (nursery plates)

For 1L NGM agar:
- 3g NaCl (ref: fisher 447300010)
- 2.5g bactopeptone (ref: BD 21167 lot 8270639)
- 17g agar (ref : A7002-5KG lot BCBM1702V)
- 1L H2O
- Salts (post-autoclave):
  25mL KH2PO4, pH=6.0 (ref : SIGMA P0662-2,5KG)
  1mL MgSO4, 7H2O (1M) (ref : SIGMA M5921-500G)
  1mL CaCl2 (1M) (ref : SIGMA C5080-500G)
  1mL cholesterol (5mg/mL) (ref : SIGMA C8667-5G)

For 1L LB broth:
- 25g LB powder (ref : fisher BP9723-500)
- 1L H2O

For 1L M9:
- 3g KH2PO4
- 7g Na2HPO4.2H2O (ref : SIGMA 71645-1KG)
- 5g NaCl

50mg/mL Kanamycin (in water, filter 0.2mm)

Antioxidants:
Trolox, NAC, Vitamin C:
- Initial concentration: 1mg/mL
- Final concentration : 200mg/mL in 4mL M9
- (pH for Trolox adjusted with NaOH)

Trans-resveratrol:
- Initial concentration: 100mg/mL 200mg in 2ml ethanol (in the freezer -20)
Intermediate concentration : 1mg/ml à in 100mL in 10ml M9 (filtered sterile)
Final concentration : 200mg/mlà in 4ml of M9 sterile, put 800ul of the intermediate solution (1mg/ml)
Put 200ul on plate (4ml NGM) to have a concentration of 10ug/ml

Trolox 238813-1G sigma (in DMSO first)
Trans resveratrol : PHR2201-200MG sigma (dilution in DMSO)
NAC, Vitamin C (dilution in water)

Goggles must be worn when operating high-power blue-light LEDs on the rig

Prepare NGM agar media and fill maintenance plates for raising N2 Bristol C. elegans on BW25113 background strain

Citation: Saul Moore Keio Acute Response Antioxidant RescueÃÂ https://dx.doi.org/10.17504/protocols.io.b3nnqmde

Preparing maintenance plates 1d

1 Make 500mL normal Nematode Growth Media (NGM) agar, following the protocol 2h

Making normal NGM for imaging plates (Cabreiro Lab)
by Saul Moore
PREVIEW RUN

2 Under a hood, pour 20ml NGM agar into each of 10 x 60mm Petri plates (maintenance plates), and leave to dry for apprximately 1 hour. Once dry, store at 4°C until seeding bacterial lawns 1h 30m

3 Inoculate an overnight culture of *E. coli* BW25113 bacteria in an Erlenmeyer flask containing 50ml LB broth, and place in at 37°C in a shaking incubator (200rpm) 20h

Inoculating a Liquid Bacterial Culture
by Priota Islam,
Imperial College London
PREVIEW RUN

4 Remove the BW culture from the shaking incubator and store at 4°C until seeding

5 Remove the maintenance plates and the BW25113 culture from 4°C storage, and leave on the bench for approximately 30 minutes to acclimate to room temperature 30m

6 Seed the maintenance plates each with 200µL of BW25113 culture, using aseptic technique working under a microbiological hood

7 Leave under hood until dry (approximately 30 min to 1 hour timing depends on humidity)

Preparing worms 2d

8 Using a platinum pick, gently pick 30 L4-stage N2 Bristol *C. elegans* onto each maintenance plate, and store in an incubator at 20°C (Monday) 1d

Citation: Saul Moore Keio Acute Response Antioxidant RescueÃÂ https://dx.doi.org/10.17504/protocols.io.b3nnqmde

9 After 24 hours, remove the adult worms, leaving the eggs behind to hatch into L1 larvae (Tuesday) 1d

10 Bleach-synchronise the worms by performing an egg prep, following the protocol: (Friday)

Egg Prep for Bleach Synchronization (Cabreiro Lab)
by Saul Moore
PREVIEW RUN

11 At around noon the next day, wash L1 larvae off the empty plate with a few mL of M9 using a glass Pasteur pipette, and re-feed onto BW-seeded maintenance plates. Incubate at 20°C. (Saturday)

Preparing bacteria

12 Prepare two Erlenmeyer flasks with 50mL LB for inoculating a liquid bacterial culture of BW control and BW25113ΔfepD bacteria. Add 50mg/mL Kanamycin to BW25113ΔfepD flask (Friday)

13 Inoculate from a single colony picked from streaked LB plates stored at 4°C, following the protocol:

Inoculating a Liquid Bacterial Culture
by Priota Islam,
Imperial College London
PREVIEW RUN

14 Leave overnight in a shaking incubator (37°C, 200rpm)

15 The next day, remove the bacterial cultures from the shaking incubator, and prepare another two Erlenmeyer flasks each with 50mL LB, for inoculating a second round of overnight cultures. Only this time, do not add Kanamycin.
(Saturday)

16 Inoculate the second round of cultures by pipetting approximately 50µL bacterial culture from

Citation: Saul Moore Keio Acute Response Antioxidant RescueÃÂ https://dx.doi.org/10.17504/protocols.io.b3nnqmde

the first overnight culture. Leave in a shaking incubator overnight (37°C, 200rpm)

17 The next day, remove the overnight cultures from the shaking incubator, and store at 4°C until seeding imaging plates the following day
(Sunday)

Preparing imaging plates

18 Make 250mL normal Nematode Growth Media (NGM) agar, following the protocol in
go to step #1
(Thursday)

Wait for the agar to cool to approximately 55°C before adding the salts, to avoid 'speckling' in imaging plates

19 Pour 3.5mL NGM agar into each well of 10 x 6-well plates, and leave to cool under a hood. Then store at 4°C until seeding lawns

20 Remove imaging plates from 4°C, and dry under a hood for 30 minutes to remove condensation. Remove the bacterial cultures from 4°C and leave on the bench for 30 minutes to acclimate to room temperature.
(Monday)

21 Pipette 30µL of bacterial culture into the centre of each well in the 6-well imaging plates, taking care not to damage the agar with the pipette tip. Seed half of the 6-well plates with BW control, and the other half with BW25113ΔfepD lawns

22 Leave the seeded plate to dry for 20 minutes under the hood, then transfer to a 25°C incubator and leave to grow for a further 7 hours and 40 minutes (for a total of 8 hours lawn growth time), before storing at 4°C for tracking the next day

23 On the day of tracking, remove the seeded plates from 4°C and dry under a hood for 30 minutes to remove condensation
(Tuesday)

Citation: Saul Moore Keio Acute Response Antioxidant RescueÃÂ https://dx.doi.org/10.17504/protocols.io.b3nnqmde

24 Prepare the antioxidants (see Materials section for details of antioxidant preparation). Pipette 200µL antioxidant solution onto the top of the lawns of each well, to yield a final concentration of 10µg/mL antioxidant (in 4mL NGM agar). Leave under a hood to dry for approximately 30 minutes

25 Leave the plates for at least 2 hours after adding antioxidants before picking worms onto the plate and tracking

Hydra Tracking

26 Prior to tracking, ensure that the imaging cave air conditioning is turned on (and there has not been a power-cut) and also empty the dehumidifier waste water tray (see pre-imaging checklist)
(Tuesday)

Normal temperature range: 19 - 21°C
Humidity: 35 - 45%

27 Remove the seeded imaging plates from 4°C and leave on bench top (with lids on) for 30 minutes to acclimate to room temperature

28 Remove the plate of age-matched (Day1 adult) worms from 20°C incubator

29 Using an eyebrow hairpick, gently but swiftly transfer 10 worms onto the edge of the bacterial lawn of each of 6 imaging plates

30 Quickly transport the 6-well plates to the imaging cave and place them under the rigs (recording the respective camera positions of each of the wells in the metadata along with the relevant treatment information)

31 Track worm behaviour on the different food + antioxidant treatment conditions for a total of 45 minutes (25 fps), applying a 10-second bluelight stimulus every 5 minutes

Citation: Saul Moore Keio Acute Response Antioxidant RescueÃÂ https://dx.doi.org/10.17504/protocols.io.b3nnqmde