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Keio Acute Response Antioxidant Rescue - Round 2

Keio Acute Response Antioxidant Rescue

Saul Moore[1]

[1]Imperial College London

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Behavioural Genomics

Saul Moore

Phenotyping the acute behavioural response of Caenorhabditis elegans (N2 Bristol) to *E. coli* single-gene deletion mutants (Keio Collection, BW25113 parent strain), 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 36 minutes, with blue-light stimulus delivered for 10 seconds at the 30, 31 and 32 minute timepoints.

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Keio Acute Response Antioxidant Rescue , Saul Moore

antioxidant, C. elegans, Keio, bacteria, behaviour, phenotype

protocol ,

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

25 x 6-well plates (imaging 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 H_2O
- Salts (post-autoclave):
  25mL KH_2PO_4, pH=6.0 (ref : SIGMA P0662-2,5KG)
  1mL $MgSO_4$, $7H_2O$ (1M) (ref : SIGMA M5921-500G)
  1mL $CaCl_2$ (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 H_2O

For 1L M9:
- 3g KH_2PO_4
- 7g $Na_2HPO_4.2H_2O$ (ref : SIGMA 71645-1KG)
- 5g NaCl

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

Antioxidants (Trolox, NAC and Vitamin C):
- Final concentration (in 4mL M9) : 500 and 1000 mg/mL
- Dilution in H_2O, except Trolox [238813-1G sigma], which was diluted in DMSO
  (pH for Trolox was adjusted with NaOH)

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

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

Preparing 6-well plates for imaging 1d

1 Make 1L 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 4mL NGM agar into each well of 25 x 6-well plates (imaging plates), and leave to dry until they lose between 3 - 5% of their original weight at pouring (approximately 2 hours). Once dry, store at 4°C until seeding bacterial lawns. 1h 30m

3 In Erlenmeyer flasks containing 50ml LB broth, separately inoculate overnight cultures of *E. coli* BW25113 (Keio Collection parent strain) and the desired bacterial mutant to test from a single colony picked from streaked LB agar plates. Add 50uL Kanamycin to the flask inoculated with the mutant bacteria. Place in a shaking incubator at 37°C (200rpm) 20h

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

4 The next day, remove the cultures from the shaking incubator and inoculate a second round of overnight cultures from the first, only this time do not add Kanamycin to the mutant bacterial culture.

5 The following day, remove the cultures from the shaking incubator and store at 4°C until used for seeding imaging plates.

6 When seeding plates, remove the plates and the cultures from 4°C storage, and leave on the bench for approximately 30 minutes to acclimate to room temperature and remove condensation 30m

7 Seed the plates each with 30µL of bacterial culture in the middle of each well of the 6-well imaging plate (using aseptic technique and working under a microbiological hood)

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8 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 BW25113 control, and the other half with BW25113ΔfepD lawns

9 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 until tracking (max 2 days)

Preparing worms 2d

10 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

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

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

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

13 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)

Imaging with worm tracking rig (Hydra)

14 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)

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

15 Remove the seeded plates from 4°C and dry under a hood for 30 minutes to remove

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condensation

16 At least 1 - 2 hours prior to adding worms and imaging, prepare the antioxidants (see Materials section for details of antioxidant preparation) and exogenously add on top of the lawns in each well, to yield a final concentration of 500µg/mL antioxidant solution (in either EtOH or H_2O) in 4mL NGM agar.

17 Leave under a hood to dry for approximately 30 minutes

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

19 Using a platinum pick, gently but swiftly transfer 10 worms onto the edge of the bacterial lawn of each well in a single imaging plate at a time

20 Quickly transport the 6-well plates to the imaging cave and place them under the rigs. Ensure that the plate is in the correct orientation for the recording so that the positions of each of the wells under the cameras is correct and matches the recorded treatment information in the metadata

21 Track worm behaviour on each well for a total of 36 minutes (at 25 fps), applying a 10-second blue-light stimulus at the 30th, 31st and 32nd minute timepoints

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