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S Keio Acute Response Antioxidant Rescue - Round 2

Keio Acute Response Antioxidant Rescue

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Behavioural Genomics



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

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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 H₂0
- 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 and Vitamin C):

- Final concentration (in 4mL M9): 500 and 1000 mg/mL
- Dilution in H₂O, 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 RUN RUN	<u> </u>
2	Under a hood, pour 4mL NGM agar into each well of 25 x 6-well plates (imaging plates), a leave to dry until they lose between 3 - 5% of their original weight at pouring (approximate hours). Once dry, store at 4°C until seeding bacterial lawns.	n 30m and :ely 2
3	In Erlenmeyer flasks containing 50ml LB broth, separately inoculate overnight cultures o <i>coli</i> BW25113 (Keio Collection parent strain) and the desired bacterial mutant to test from 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)	f <i>Ê.</i> m a
	Inoculating a Liquid Bacterial Culture by Priota Islam, Imperial College London	<u> </u>
4	The next day, remove the cultures from the shaking incubator and inoculate a second ro overnight cultures from the first, only this time do not add Kanamycin to the mutant bact culture.	

- 5 The following day, remove the cultures from the shaking incubator and store at 4°C until used
- 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
- 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)

for seeding imaging plates.

- 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)

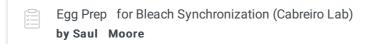
Preparing worms

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



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%

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



5

condensation

- 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 H₂O) in 4mL NGM agar.
- 17 Leave under a hood to dry for approximately 30 minutes
- 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
- 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