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Behavioural phenotyping of *C. elegans* on UV-killed *E. coli* mutants

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Behavioural Genomics

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Protocol for screening candidate behaviour-modifying *E. coli* BW25113 single-gene deletion mutants from the 'Keio Collection', to investigate their effects on *Caenorhabditis elegans* behaviour when killed by ultraviolet (UV) light

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12 x Whatman Square Well Flat Bottom UNIPLATE, 7701-1651
25 x ThermoFisher Scientific Nunc™ 96-Well Polystyrene Round Bottom Microwell Plates, Non-Treated, 268200

100 x 60mm Petri plates
3 x 90mm Petri plates
3 x 150mm Petri plates
2 x 50mL Erlenmeyer flask

1 x 96-pin replicator
500mL LB broth media
1L NGM agar (for ingredients, see protocol for making NGM agar)

Preparing NGM agar + pouring plates

1 Prior to screening, prepare the materials needed for screening C. elegans on selected Keio E. coli mutants (9 candidate mutants + wild-type BW control). For a single experiment replicate (10 biological replicates of each mutant, screened in 2 runs with the laboratory's 'Hydra' imaging rig):

- 12 Whatman 96-square-well flat-bottom plates ('imaging plates')
- 25 Nunc™ 96-round-well round-bottom microwell plates ('culture' plates)
- 3 x 150mm Petri plates ('nursery plates')
- 3 x 90mm Petri plates ('maintenance plates')
- 100 x 60mm Petri plates ('uv-killing plates')
- 110 x 15mL Falcon tubes
- 2 x 50mL Erlenmeyer flasks

2 Make 1L normal Nematode Growth Media (NGM) agar, following the protocol:

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

3 Pour 20ml NGM agar into each maintenance plate, and 50ml NGM agar into each nursery plate, following the protocol for Plate pouring (dx.doi.org/10.17504/protocols.io.6bhhaj6).

Keep the remaining agar warm in a water bath set to 65°C, for dispensing into 96-well imaging plates afterwards

4 Using the Integra ViaFill, dispense 200µL of NGM agar into each well of the 10 imaging plates,

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following the protocol:

Dispensing agar into multiwell plates
by Saul Moore

PREVIEW RUN

5 Leave the plates on the lab bench (with lids on) until the agar has cooled and solidified (approximately 1 hour, timing depends on humidity)

6 Measure the weight of 3 imaging plates (with lids on) and record average plate weight on day of pouring

7 Dry the imaging plates under a hood (or drying cabinet) until the plates lose between 3-5% of their original plate weight (with lids on)

8 Store the imaging plates upside-down at 4°C until used for experiments

Seeding Petri plates + worm maintenance

9 Inoculate 10ml LB broth media with E. coli BW25113 (Keio background wild-type strain, used as negative control and for raising worms, no Kanamycin) in an Erlenmeyer flask for overnight culture following the protocol:

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

PREVIEW RUN

10 Place the inoculation in a shaking incubator at 37°C at 200 rpm and leave to grow overnight

11 Remove the BW culture from the shaking incubator and place in 4°C fridge until seeding

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12 Remove the plates from storage and the BW culture from the fridge, and leave on the bench for approximately 30 minutes to acclimate to room temperature

13 Using aseptic technique, seed the maintenance plates each with 400µL of BW25113 culture

14 Leave under hood until dry (with lids on, timing depends on humidity)

15 Using a platinum pick, gently pick 30 adult N2 Bristol C. elegans onto each maintenance plate, and store in an incubator at 20°C
(Monday)

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

17 Inoculate a further 10ml LB broth media with BW25113 bacteria for overnight culture, following the protocol in **go to step #9** and place in a shaking incubator at 37°C, 200 rpm
(Wednesday)

18 After 24 hours, remove the culture from the incubator, and the nursery plates from storage, and leave to acclimate on bench top for 30 minutes
(Thursday)

19 Seed the nursery plates each with 1mL of fresh BW25113 culture. Leave under hood until dry

20 Wash the worms off the BW-seeded maintenance plates, into two 15ml Falcon tubes
(Friday)

21 Perform an egg prep on worms in the Falcon tubes, following the protocol:

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

PREVIEW RUN

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22 At around noon the next day, wash L1 larvae off the empty plate and re-feed onto the BW-seeded nursery plates using a glass Pasteur pipette. Aim to dispense around 3000 worms per plate. Incubate at 20°C
(Saturday)

Inoculating from frozen stocks (96-well)

23 Remove the required stock plates from -80°C containing the selected candidate strains. Gently remove the aluminium film and leave to partially thaw for a minute or so

To avoid damaging the bacterial stocks through repeated freeze-thawing, do not let the wells completely defrost. Just enough to be able to pick up some cells with the replicator.

24 Inoculate individual vials containing 4mL LB broth and 50µg/ml Kanamycin from the selected wells of the Keio frozen stock plates, following the protocol:

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

PREVIEW RUN

25 Wet some tissue with MilliQ water, wrap the culture plates in the tissue, and incubate overnight at 37°C (no shaking)

The tissue provides humidity that aids growth, while the presence of Kanamycin should prevent contamination

26 Also inoculate 10mL LB broth media in an Erlenmeyer flask with BW control, and place in a shaking incubator overnight at 37°C, 200 rpm

27 Using a multi-pipette, fill half of wells (those designated for live bacterial cultures) of 10 x 96-well culture plates with 200µL LB broth (as per the desired plate layout). Fill those wells with 50µg/mL Kanamycin, except for the wells that are reserved for BW control.
(Wednesday)

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28 Remove the overnight cultures from the incubator. Using a sterile pipette tip (or inoculation loop), inoculate the wells of the culture plates with strains from the overnight culture vials designated for live culture. Inoculate the wells without Kanamycin with the BW control.

29 Optional: Make a template stock plate for -80°C storage (live strain layout only): mix 200µL culture with 15% glycerol in each well

30 Fill another round of individual 15mL Falcon tubes each with 4mL fresh LB broth, for overnight culture of strains destined for UV-treatment. Add 50µg/mL Kanamycin to all tubes except those reserved for the UV-treated BW control.

31 For strains designated for UV-treatment, inoculate the new Falcon tubes from the previous overnight culture, following the above protocol in **go to step #24**

32 Incubate both the live cultures in 96-well format (no shaking) and the cultures for UV-treatment in vials (shaking) overnight at 37°C

33 Remove the overnight cultures from the incubator. Again, fill half of the wells of 10 culture plates (designated for live bacteria) with 200µL LB broth. This time do not add Kanamycin. (Thursday)

34 Inoculate the second round of overnight cultures from the first in 96-well format (for live bacteria), using a 96-pin replicator, following the protocol:

Growing overnight bacterial culture in 96WP
by Priota Islam,
Imperial College London
PREVIEW RUN

35 Fill another round of 15mL Falcon tubes with 4mL fresh LB broth for the second round of inoculations of the overnight cultures in vials for UV-treatment (no Kanamycin)

36 Inoculate the new vials from the previous overnight cultures, by following the above protocol for *Inoculating a Liquid Bacterial Culture* **go to step #24**

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37 Place the 96-well culture plates (no shaking) and the vials (shaking) in an incubator for overnight culture at 37°C

UV-killing bacteria

38 Clean the CL-1000 Ultraviolet crosslinker machine by wiping down with distilled MilliQ water and 70% ethanol. Turn on the UV light and leave for 5 minutes to decontaminate
(Friday)

39 Remove the overnight cultures in vials from the incubator, add 4mL fresh LB broth to each culture vial (total 8mL) and pour into empty 60mm plates for UV-killing
(10 replicates for each strain tested; 10 strains = 100 plates)

40 Place the plates inside the machine, and remove their lids

41 Expose the bacterial cultures to UV light (365nm wavelength) for 10 minutes

42 Remove the plates from the machine, replace the lids, and leave to stand for 5 minutes

43 Repeat steps **go to step #40** to **go to step #42** six more times, to ensure that the bacteria are dead

This process may need to be repeated (in batches of up to 25 plates) due to maximum capacity of the UV machine

44 Transfer the bacterial cultures to separate 15mL Falcon tubes, and top up to 15mL with LB broth

45 Centrifuge the bacteria for 10 minutes at 4,000 rpm to pellet the bacteria at the bottom of the tubes

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46 Remove the supernatant using a plastic Pasteur pipette, and store at 4°C

Seeding imaging plates (96-well)

47 Remove the imaging plates from 4°C storage and record the average weight of 3 randomly selected plates
(Friday)

48 Ensure that imaging plates have lost approximately 3-5% of their original weight. Place under a hood or drying cabinet until they have

49 Remove overnight cultures of live Keio strains and the pelleted dead Keio strains from 4°C storage

50 Re-suspend the bacteria by adding 3mL LB broth and vortexing

51 Add 200µL of re-suspended dead bacterial culture to the empty wells of the overnight culture plate with live bacteria, to complete the experimental plate layout, with an equal proportion of wells with live bacterial cultures and wells with dead bacterial cultures

52 Using the Integra ViaFlo, seed 10µl of bacterial culture from the wells of each live overnight culture plate into the corresponding wells of each imaging plate

Ensure correct plate orientation under the Integra ViaFlo, with well A1 in the top left corner

53 Place seeded plates under a hood to dry for 20 minutes, then place in an incubator at 25°C (no shaking) for 7 hours 40 minutes (total lawn growth time: 8 hours)

54 After 8 hours, remove the plates from the incubator and store at 4°C

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COPAS worm-sorting + Hydra tracking (96-well)

55 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%

56 Remove the nursery plates from the incubator. Wash the worms off the plates into two 15ml Falcon tubes using approximately 10mL sterile PBS 'A' buffer

57 Fill up the tubes to 15ml with PBS 'A' and centrifuge at 1000rpm for 2 minutes

58 Remove the supernatant using a Pasteur pipette

59 Repeat steps **go to step #57** to **go to step #58** four more times to thoroughly rinse off any remaining control BW25113 bacteria

60 Re-suspend the worms and divide them equally into two 50ml Falcon tubes (for the COPAS), and fill them both up to approximately 40ml with PBS 'A'

61 Use the COPAS to dispense three Day1 adult worms into each well of the 10 imaging plates, following the protocol:

COPAS wormsorter v.2
by Bonnie Evans
PREVIEW RUN

62 Leave the plates to dry under a hood for 30 minutes to 1 hour (until dry, timing depends on humidity), then place in incubator at 20°C until tracking (at +4 hours on food)

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Check that worms are crawling (not swimming) on plates, and lawns appear matt in colour (not wet)

63 30 minutes prior to tracking with the Hydra rig (every 20 minutes, 2 runs in total), remove 5 imaging plates from the 20°C incubator and leave to acclimate in the imaging cave

64 Record worm behaviour on the bacterial food for 15 minutes at the 4-hour timepoint (25 fps, exposure: 25000 msec, blue-light stimulation)

65 After tracking, discard the plates in a biological waste bin

66 Check tracking checklist to ensure that all videos have been saved correctly: '/Volumes/behavgenom$/Documentation/Protocols/analysis/tracking-checklist-20210210.docx'

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