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Screening effects of excess copper on worm behaviour

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ABSTRACT

Protocol for tracking the behaviour of mutant (cua-1[H828Q], Wilson's Disease ortholog) vs wild-type (N2) worms: in the absence of treatment; exposed to 25-100uM $CuCl_2$ for 4h; reared for one generation on NGM+$CuCl_2$ plates (F1s); and reared for two generations on NGM+$CuCl_2$ plates (F2s).

Pick L4 worms for bleaching (9 days prior to tracking)

1 Pick 10 x L4 worms onto 10 x 90mm NGM-agar plates pre-seeded with *E. coli* OP50 for each strain to be tested. In this protocol we are comparing the response of a mutant strain containing

a H828Q mutation in the *cua-1* gene, cua-1[H828Q], to WT control (N2).

Therefore 20 x 90 mm plates will be picked in total.

Pour 150mm nursery plates (up to 6 days prior to tracking)

2 Prepare 4.5L NGM-agar and autoclave.

Protocol

NAME

Making normal NGM

CREATED BY

Bonnie Evans

PREVIEW

3 Meanwhile prepare a 1 M stock solution of copper chloride ($CuCl_2$) by dissolving 134.45mg of powder in 1mL of sterile deionised water.

Copper (II) chloride dihydrate **Fisher Scientific Catalog #10125-13-0**

4 Sterilise by passing through a 0.22 micron syringe filter into a clean microfuge tube wrapped in tinfoil.

5 Once agar has cooled to around 62°C , add the post-autoclave salts and cholesterol (as described in the protocol above).

6 Label 4 x pre-sterilised 1L bottles (red screw cap lids from media kitchen) with desired concentration of $CuCl_2$ to be tested (i.e. 100;75,50;25µM).

Aseptically transfer 1L of NGM to each of these bottles. I recommend doing this in the microbial hood that has been throughly wiped down with 70% EtOH/Distel prior to use.

7 Aseptically add $CuCl_2$ from stock to desired concentration, if preparing 1L solutions and 1M stock (as above):

100uL stock solution [1M] in 1L NGM = 100µM final conc
75uL stock in 1L = 75µM
50uL stock in 1L = 50µM
25uL stock in 1L = 25µM

8 Here we want to look for behavioural differences between the mutant and control for the first generation (F1) and second generation (F2) worms reared on various concentrations of copper. Since we will be doing 3 day replicates, we will need 12 x 150 mm plates for each concentration to be tested.

Hence, pre-label 12 x 150mm petri dishes (nursery plates) for each concentration of copper.

The remaining 0.5L of NGM will be used for 'no compound/no treatment' control plates (only 6 needed in total).

9 Pour 75mL of agar into the respective nursery plates and leave to dry in the hood.

Protocol

NAME
Plate Pouring

CREATED BY
Priota Islam

PREVIEW

10 Once dry, invert plates to be agar-side up and place in an opaque/tin foil-lined air tight container. Store at 4°C.

Pour tracking plates (up to 4 days prior to tracking)

11 Prepare 1L of no-peptone NGM and autoclave.

Protocol

NAME

Making no peptone NGM for imaging plates

CREATED BY

Bonnie Evans

PREVIEW

12 Once agar has cooled to around 62°C , add the post-autoclave salts and cholesterol (as described above).

13 Meanwhile make up fresh stocks of 1M $CuCl_2$ (as described above).

14 Label 4 x pre-sterilised 250mL bottles (red screw cap lids from media kitchen) with the concentrations of $CuCl_2$ to be tested (i.e. 100;75,50;25µM).

As above, aseptically transfer 200mL of NGM to each of these bottles and add $CuCl_2$ to the desired final concentration.

15 Pre-label 9 x tracking plates with each concentration of $CuCl_2$. This will allow for 3 day replicates of: 4h exposure to excess copper, F1 worms and F2 worms to be tracked on separate plates.

Label 3 x tracking plates as 'NGM only' for the no compound controls.

16 Dispense agar into square 96-well plates using VIAFILL dispenser. Dispense 200 µL per well. Once cooled, store agar side up (plate lids on) in an opaque/tinfoil lined airtight container at 4°C.

Protocol

NAME

Dispensing agar into multiwell plates

CREATED BY

Ida Barlow

PREVIEW

Dry plates and bleach worms (5 days prior to tracking F1 w...

17 Dry 2 x 150 mm NGM-agar plates of copper concentration (i.e. 0;25;50;75;100µM, with 1 plates for mutant and control) in the drying cabinet (setting 2) for 3 hours.

18 Seed 150 mm plates with *E. coli* OP50 and leave to dry on bench at room temperature overnight.

19 Bleach synchronise worms prepared in step 1 and leave in diapause for 2 days at 20°C on a rotator that is constantly spinning.

Protocol

NAME

Bleach synchronisation of C. elegans

CREATED BY

Ida Barlow

PREVIEW

Refeed L1s (3 days prior to tracking F1 worms)

20 Pre-label the pre-seeded 150mm plates with the relevant strain name and date, i.e. 'N2' or 'cua-1[H828Q]', for each copper concentration, remember to include a no compound control.

21 At 16:45, spin L1s (prepared in step 7) for day 1 of tracking using centrifuge program 1 (1500 rpm for 2 mins).

22 Use a 3 mL plastic pasteur pipette to carefully remove the supernatant (leaving ~1 mL) then resuspend the worm pellet by gently flicking the tube.

23 At 17:00, use a clean rubber teat and glass pipette per strain, carefully aspirate the L1 worm suspension and drop 4 small droplets onto the bacterial lawn (~1000-1500 worms total) of the pre-seeded/pre-labelled 150mm nursery plates.

24 Incubate, agar-side down, at 20°C for 15 mins to allow the droplets containing L1 worms to dry.

25 Flip plates to be agar-side up and incubate at 20°C.

Dry and seed tracking plates (1 day prior to tracking F1 wo...

26 In the morning, remove 2 x square 96-well tracking plate from the cold room per copper concentration and weigh three random plates without their lids.

27 Allow plates to dry, with lids off, in the drying cabinet (setting 2) until they have reduced in weight by 3-5% (~1.5 - 2 hours).

28 Pre-label dried tracking plates (step 10) with the imaging run and drug information, so that every plate on a single day of imaging has a unique plate id. For example 100µM_F1_run1, where:

- '100µM' is the concentration of copper on the plate
- '_F1' stands for F1 worms reared on copper from the point of refeeding (use '4h' for 4h exposure)
- '_run1' is the imaging run number that day

29 Ensure optical density of *E. coli* OP50 stock is ~1.0 (an OD of 0.96 - 1.04 is acceptable).

30 Use the VIAFILL dispenser to seed all the tracking plates with 5 µL bacterial suspension per well and leave to dry (~20 mins).

31 Flip tracking plates to be agar-side up, cover with an opaque box and leave at room temp overnight.

Dry and seed F2 nursery plates (1 day prior to tracking F1 w...

32 Prepare nursery plates for rearing the second generation of worms (F2) on increased copper concentrations by repeating steps 17-18.

Again, use 1 x 150mm plate for WT and mutant strain. Ensure that you clearly labelled the plates with the concentration of copper, worm strain and generation number.

Tracking F1 worms and worms exposed to excess copper fo...

33 At 09:00, wash young adult worms off the 150 mm plates using M9 buffer, and a clean 3 mL pasteur pipette per strain, into 15 mL falcons (labelled with strain name and copper treatment).

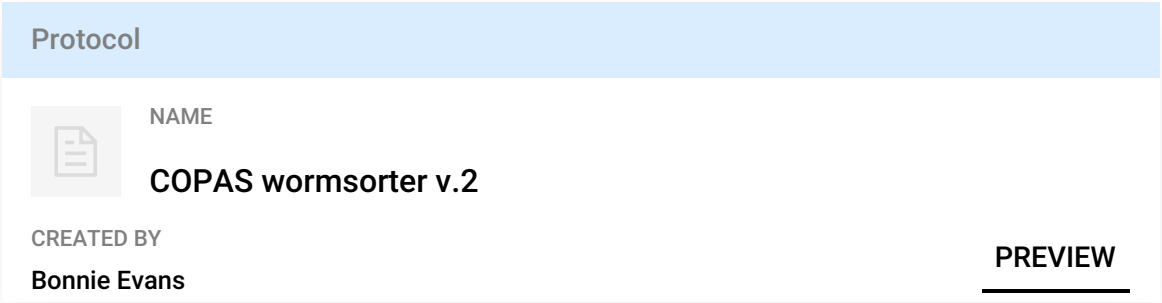
34 Centrifuge using program 1 (1500 rpm for 2 mins) to pellet the worms.

35 Carefully remove and discard the supernatent and refill with M9 .

36 Repeat steps 33-34.

37 After final wash, fill 15 mL falcon with M9. Transfer contents to a clean 50ml falcon and top up to 45 mL with M9.

38 Use COPAS wormsorter to dispense 3 worms per well (pure, no double) into their respective imaging plates, filling 5 plates at a time.



38.1 To test the effects of exposing worms to copper for 4h simply dispense worms from the NGM only nursery plates into the wells of tracking plates containing the desired concentration of copper.

In this experiment I fill half of the plate with WT and half with cua-1[H828Q]

39 Allow liquid to dry by placing imaging plates in microbial safety cabinet (pre-sterilsed with 70% ethanol) with lids off for ~30 minutes.

40 Use a microscope to check that wells are dry and incubate, agar-side up, in the 20°C incubator.

Track worms incubated on the tracking plates after 4 hours, as calculated from the median point

41 of dispensing worms (middle wormsorter time), and allow worms 30 mins to acclimatise to conditions in the tracking room prior to imaging.

For example:
- Wormsorter start time = 10:00
- Wormsorter end time = 10:40
- Middle wormsorter time = 10:20

- Place in tracking room at 13:50
- Start imaging run at 14:20

42 Image on the multi-camera tracker (Hydra) using 'run_syngenta_experiment_v2.py' protocol script:

1) 5 min pre-stimulus recording
2) 6 min blue light recording: 60 sec no light, [10 sec light ON, 90 sec light OFF] x 3 (6 min total)
3) 5 min post-stimulus recording

Preparing F2 worms (Day of tracking F1 worms)

43 While tracking plates are drying, recover the 50mL falcon tubes containing worms reared on various copper concentrations and transfer remaining worm suspension to fresh 15mL falcon tubes.

44 Centrifuge using program 1 (1500 rpm for 2 mins) to pellet the worms.

45 Use a 3 mL plastic pasteur pipette to carefully remove the supernatant (leaving ~1 mL) then resuspend the worm pellet by gently flicking the tube.

46 Refeed ~50 gravid worms onto fresh nursery plates containing the same concentration of copper they have been reared on.

As before, allow droplets to dry and incubate, agar-side up, at 20°C overnight.

Preparing F2 worms (1 day after tracking F1 worms)

47 The F1 worms refed yesterday will have now laid their eggs onto the F2 nursery plates. Use an eyelash pick to remove these adult worms from the plates, continue incubating the nursery plates at 20°C monitoring their development.

Dry and seed tracking plates (1 day prior to tracking F2 wo...

48 Repeat steps 26-31 to prepare plates for tracking the behaviour of the second generation of worms reared on plates containing excess copper.

Tracking F2 worms

49 Repeat steps 33-42 to track the F2 worms.

50 Transfer videos to BehavGenom and analyse with Tierpsy.