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Ataxia Mutant mca-3 Drug Repurposing Screen

Forked from unc-80 LoF Mutant Drug Repurposing/Confirmation Screening

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Behavioural Genomics

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We use this protocol and it's working

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Abstract

Protocol for conducting a drug screen of the SPECS Drug Repurposing Library (5632 compounds) to find drugs that rescue the "ataxic" phenotype of adult *mca-3(ar493) C. elegans* mutants. Forked from *unc-80* drug screening protocol (by Thomas J. O'Brien). This screen tests adult hermaphrodite *C. elegans* behaviour at 5hrs and 20hrs of drug exposure. Each 96-well plate contains 88 wells with unique compounds at 25uM and 8 wells with DMSO (solvent) as a negative control. Each recording day also contains a control plate of N2 worms on DMSO. For ease of recording, the 64-plate library was split into two sets of 32 plates, meaning each recording day had 32 plates of *mca-3(ar493)* on drugs/DMSO and one plate of N2 on DMSO (33 plates/day). Plates were seeded with FPA-killed OP50 with a higher OD of ~1.8, allowing for testing at 20hrs without the worms running out of food.

Pick worms for bleaching (9 days prior to tracking)

1 Pick or scoop ~30 x *mca-3(ar493)* worms onto 20 × 90mm NGM-agar plates, and 2 x L4 N2 (wild-type) worms onto 2 × 90mm NGM-agar plates pre-seeded with *E. coli* OP50 and incubate at 20°C.

In this screen we are looking for compounds that rescue the behaviour of the *mca-3(ar493)* RoF mutant (pushing it in phenomic space towards N2)- therefore we need lots of *mca-3(ar493)*worms and just a few N2 worms for controls. The *mca-3(ar493)* worms have a lower brood size along with slow growth (1 extra day needed for adulthood) and need more worms for bleaching compared to N2.

Pour 96-well tracking plates and 150 mm nursery plates (up to 4 days before tracking)

2 For the whole compound library screen we require 65 *imaging plates*, therefore prepare 1.5 L no peptone NGM-agar.

Protocol

NAME

Making no peptone NGM for imaging plates

CREATED BY

Bonnie Evans

Preview

3 Once agar has cooled to around 62°C , add the post-autoclave salts and cholesterol then 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 airtight container at 4°C.

Protocol

NAME

Dispensing agar into multiwell plates

CREATED BY

Saul Moore

Preview

4 We also require fourteen (14) 150 mm nursery plates to grow up the synchronized worms. These plates will be seeded with live OP50, so should have peptone. Prepare 1L NGM agar and add post-autoclave salts and cholesterol as normal.

Seed plates and bleach worms (5 and 6 days prior to tracking)

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

6 **6 days before tracking**, bleach synchronise *mca-3(ar493)* worms prepared in step 1 and leave in diapause for 2 days at 20°C on a rotator that is constantly spinning. The *mca-3(ar493)* require an extra day to reach adulthood and should be prepared always one day before the N2 controls.

Protocol

NAME

Bleach synchronisation of C. elegans

CREATED BY

Ida Barlow

Preview

7 **5 days before tracking**, bleach synchronise N2 worms prepared in step 1 and leave in diapause for 2 days at 20°C on a rotator that is constantly spinning.

Refeed L1s (3 and 4 days prior to tracking)

8 **Four days before tracking**, at 17:45, spin *mca-3(ar493)* L1s (prepared in step 6) for day 1 of tracking using centrifuge program 1 (1500 rpm for 2 mins). *mca-3(ar493)* worms need an extra day to reach adulthood.

9 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.

10 Label twelve (12) 150 mm seeded plates with the strain *mca-3(ar493)* and the date and time of refeeding.

11 At 18:00, use a clean rubber teat and glass pipette carefully aspirate the *mca-3(ar493)* L1 worm suspension and drop 4 small droplets onto the bacterial lawn of twelve (12) of the pre-seeded/pre-labelled 150mm nursery plates.

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

13 Flip plates to be agar-side up and allow to grow at 20°C.

14 **Three days before tracking**, repeat steps 8-13 for N2 worms onto two (2) 150 mm plates, remembering to note the day and time of refeeding.

Dry, dose and seed tracking plates (1 day prior to tracking)

15 In the morning, remove 33 square-well imaging plate from the cold room (prepared in steps 2-3) and weigh three random plates without their lids.

In this screen, the SPECS library (64 plates) was split into two, to test half the library (32) during each experimental bout. In each bout, there was also an additional plate entirely filled with N2s on DMSO totalling 33 plates total per experimental bout. The experimental bouts included a tracking session at 5hrs and another at 20hrs after initial drug exposure.

16 Allow plates to dry, with lids off, in the drying cabinet (setting 2.5) until they have reduced in weight by 4-5% (~2 hours).

17 Create 33 new entries in Airtable under the "Plates" tab. This should generate a unique plate ID for each tracking plate. Pre-label dried tracking plates with the SPECS ID and Airtable plate ID, so that every plate on a single day of imaging has a unique label. For

example: SPECS_04 plate_895. Upload the corresponding metadata excel sheet to the "Well Information" column in Airtable → Plates.

18 Remove the drug library from the -80°C freezer five plates at a time, and allow the first 5 plates to thaw at room temperature.

Note: *Compounds from the SPECS library came pre-dissolved in DMSO at a concentration of 10mM, in 96-well plate format. These were diluted 1:4 in DMSO onto 96-well drug source plates for a concentration of 2.5mM.*

19 Working one plate at a time (so that plates don't dry before drugs are added), use the VIAFILL (with the small cassette) to dispense 5 µL of sterile deionised water onto the corresponding pre-dried and labelled imaging plate.

20 Use VIAFLO on the 'TAY2' custom program to transfer 2.05 µL of diluted drug and DMSO mixture onto the corresponding pre-labelled tracking plate [position B of VIAFLO].

This uses the formula below to perform a further 1:100 dilution of the compound, i.e. the 2.5 mM drug stock → 25 µM final imaging plate concentration:

$$\frac{\text{Volume of diluted drug stock } (2.05\mu L)}{\text{Volume of agar } (200\mu L) + \text{Volume of water } (5\mu L)} \times \text{ Diluted drug stock concentration } (2.5mM) = \text{Final drug concentration } (25\mu M)$$

21 Repeat steps 18-20 until all tracking plates have been dosed with the relevant compounds.

22 Ensure optical density of concentrated PFA-killed OP50 bacterial stock is ~1.8 (an OD of 1.75 - 2.0 is acceptable).

Note: When making the PFA-killed bacteria, on the final wash, add 25mL M9 instead of 50mL M9 to concentrate the bacterial stock and obtain a higher OD. This higher OD is required for the 20 hr drug timepoint -- without it, the worms would run out of bacteria before reaching 20 hrs.

Protocol:
Making PFA-killed bacteria

23 Use the VIAFILL dispenser to seed all the tracking plates with 15 µL bacterial suspension per well and leave to dry (~20 mins).

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

Tracking

25 At 11:00, 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.

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

27 Carefully remove and discard the supernatant and refill with M9.

You can either remove the supernatant with a bulb pipette or with the Integra Vacusafe fitted with a glass pipette.

28 Repeat steps 26-27.

29 After final wash, fill each 15 mL falcon with M9. Transfer contents to a clean 100mL glass lab bottle labelled with the strain name *mca-3(ar493)* or N2 on the side.

30 Mix/swirl the worm solution and place two drops of 100 uL of worm suspension on an empty petri dish or other glass surface. Estimate the number of worms swimming in each 100 uL drop and divide by 10 to get the worm density per 10 uL. If too concentrated, add an appropriate amount of M9. If too diluted, spin again (program 1), remove M9, and re-count worm suspension.

31 Use the VIAFILL to dispense 10 uL of worm solution per well of the pre-dried, seeded, 96-well imaging plate using the large cassette.

32 Allow liquid to dry by placing imaging plates in microbial safety cabinet (pre-sterilsed with 70% ethanol) with lids off for ~60 minutes.

While plates are drying is a good time to clean the tracking lids and ensure there is no dust on them.

33 Use a microscope to check that wells are dry and incubate, agar-side up, in the microbial safety cabinet.

34 Track the behaviour of worms incubated on the drugs after 5 hours, as calculated from the median point of dispensing worms, and allow worms 30 mins to acclimatise to conditions in the tracking room prior to imaging.

For example:
- Worm dispensing start time = 11:50
- Worm dispensing end time = 12:10
- Middle dispensing time = 12:00

5 hour exposure to drugs:
- Place in tracking room at 16:30
- Start imaging run at 17:00

20 hour exposure to drugs:
- *Worms already in tracking room*
- Start imaging run at 08:00

35 Use Airtable → Runs to image on the multi-camera tracker (Hydra) using 'run_syngenta_experiment_v2.py' (script 15) 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

Note: each plate should be one "run" in Airtable → Runs

36 Transfer videos to BehavGenom and analyse with Tierpsy.

37 Keep the worms overnight in the imaging room underneath an opaque box. Repeat steps 34-36 at 20 hours.