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We use this protocol and it's working

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# unc-80 LoF Mutant Drug Repurposing/Confirmation Screening

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Behavioural Genomics

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ABSTRACT

Combined protocol for conducting a drug repurposing screen of the MRCT FDA-approved compound library (743 compounds) to find drugs that rescue the behavioural phenotype of adult *unc-80*(*syb1531*) *C. elegans* mutants, or the following confirmation screen using a subset of 30 potential hits. Both screens follow the same timeline (i.e. 4h exposure of worms to drugs), but the initial screen simply has more drug plates and fewer well replicates compared to the confirmation screen.

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

1 Pick 10 x L4 *unc-80(syb1531)* worms onto 20 x 90mm NGM-agar plates, and 10 x L4 N2 (wild-

type) worms onto 2 x 90mm NGM-agar plates pre-seeded with *E. coli* OP50 and incubate at 20°C. *(22 plates will be picked in total)*

In this screen we are looking for compounds that rescue the behaviour of the *unc-80* LoF mutant (pushing it in phenomic space towards N2)- therefore we need lots of *unc-80 worms* and just a few N2 worms for controls.

## Pour 96-well tracking plates (up to 4 days before tracking)

2 For the whole compound library screen we require *90 imaging plates*, therefore prepare 2 L no peptone NGM-agar.

For the hit validation/confirmation screen we require *15 imaging plates*, therefore prepare 500 mL 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

## Dry plates and bleach worms (5 days prior to tracking)

4 Dry 12 x 150 mm NGM-agar plates per strain (10 plates for the *unc-80* mutant and 2 plates for N2) in the drying cabinet (setting 2) for 3 hours.

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

6 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)

7 Pre-label the 12 pre-seeded plates (prepared in step 4-5) with the relevant strain name and date, i.e. 10 x plates labelled "*unc-80* LoF" and 2 x plates labelled "N2".

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

7.2 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.

7.3 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 of the pre-seeded/pre-labelled 150mm nursery plates.

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

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

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

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

*For the whole compound library screen: X = 30 plates*
*For the confirmation screen: X = 5 plates*

9 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).

10 Pre-label dried tracking plates (step 9) with the imaging run and source plate information, so that every plate on a single day of imaging has a unique plate id. For example Sell04_rep2_run3, where:
- 'Sell04' corresponds to the fourth source plate of the SelleckChem drug library
- '_rep2' is the second day replicate of that source plate
-'_run3' means this plate will be tracked in the third imaging run

11 Remove the drug library from the -80°C freezer and allow to thaw at room temperature.

Note: *For both screens, the MRCT SelleckChem compound library (and a resupply of hits) was provided by LifeArc. Compounds came pre-dissolved in DMSO at a concentration of 10mM, in 96-well plate format.*

12 Briefly spin drug source plates in the mini bench-top plate spinner (~15 seconds) to collect all

plate contents at the bottom of the wells.

13 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 a pre-dried imaging plate (step 9).

14 Use VIAFLO on the 'Tom_BG_Agar' custom program to transfer 2.05 µL of diluted drug and water mixture onto the corresponding pre-labelled tracking plate [position A of VIAFLO].

This uses the formula below to perform a further 1:100 dilution of the compound, i.e. the 10 mM drug stock -> 100 µM final imaging plate concentration:

$$\frac{Volume\ of\ diluted\ drug\ stock\ (2.05\ \mu L)}{Volume\ of\ agar\ (200\ \mu L) + Volume\ of\ water\ (5\ \mu L)} \times Diluted\ drug\ stock\ concentration\ (10\ mM) = Final\ drug\ concentration\ (100\ \mu M)$$

15 Repeat steps 13-14 until all tracking plates have been dosed with the relevant compounds.

16 Ensure optical density of OP50 bacterial stock is ~1.0 (an OD of 0.96 - 1.04 is acceptable).

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

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

## Tracking

19 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 (wash one plate into one falcon tube to prevent worm density being too high and blocking the COPAS).

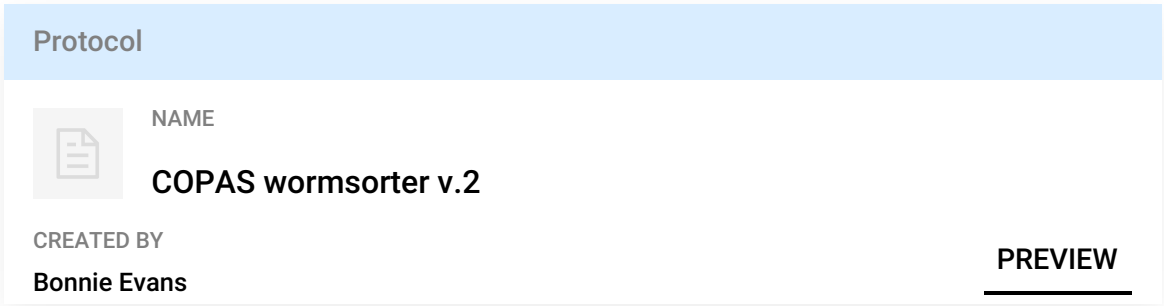
20 Centrifuge using program 1 (1500 rpm for 2 mins) to pellet the worms.

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

22 Repeat steps 20-21.

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

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




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

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

*them.*

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

27 Track the behaviour of worms incubated on the drugs after 4 hours, as calculated from the median point 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

4 hour exposure to drugs:
- Place in tracking room at 13:50
- Start imaging run at 14:20

28 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

29 Transfer videos to BehavGenom and analyse with Tierpsy.