MAR 21, 2023

OPEN ACCESS

DOI:
dx.doi.org/10.17504/protocols.io.dm6gpj19dgzp/v1

Protocol Citation: Thomas J O'Brien 2023. Reticulon Mutant Drug Screen. **protocols.io** https://dx.doi.org/10.17504/protocols.io.dm6gpj19dgzp/v1

Protocol status: Working
We use this protocol and it's working

Created: Mar 20, 2023

Last Modified: Mar 21, 2023

PROTOCOL integer ID:
79113

Reticulon Mutant Drug Screen

Thomas J O'Brien[1]

[1]Imperial College London

Thomas J O'Brien
Imperial College London

ABSTRACT

Protocol for comparing the effects of various compounds on the phenotype our *ret-1* (*RTN2* ortholog) LoF mutant vs N2 control.

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 case we are comparing the response of our *ret-1* LoF mutant with N2

(parental control), therefore 20 x 90 mm plates will be picked in total.

Prepare drug source plates (up to 3 days before first day of..

2 Prepare 96-well source plates with stocks of all the compounds and concentrations to be tested.

Note: It is best to shuffle around the position of compounds within the source plate to overcome any potential edge effects

2.1 Weigh out compounds using a micro balance and dissolve in an appropriate solvent (sterile water or DMSO) to achieve x1000 the highest concentration that will be tested (this accounts for dilution of compounds when adding to the tracking plates).

For example, to test at a compound at a maximum concentration of 100 µM, a 100 mM top stock of the compound is required

2.2 Perform a 10-fold serial dilution of the top-stock of each compound, using the same solvent initially used to dissolve the compounds.

For example, mix 50 µL 0.1 M compound top stock with 450 µL solvent and vortex (yielding 0.01 M concentration)

2.3 Repeat previous step (using the newly generated dilute compound) to obtain all desired concentrations to be tested.

2.4 Dispense 50 µL of the drug stocks into the relevant wells of a 96-well v-bottomed microtitre plate. Remember to include control wells (i.e. drug solvent only) and label the plates (unique plate ID and date) with a frost-proof marker pen.

2.5 Use a plate seal to seal source plates, and store at -20°C until use.

For this experiment 3 unique source plates were generated

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

3 Prepare 2 L no peptone NGM-agar and autoclave.

Protocol

NAME

Making no peptone NGM for imaging plates

CREATED BY

Bonnie Evans

PREVIEW

4 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

For this experiment 24 tracking plates are imaged per day: pour 75 plates total

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

5 Dry 14 x 150 mm NGM-agar plates per strain (7 plates for *ret-1* mutant and 7 plates for N2) in the drying cabinet (setting 2) for 3 hours.

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

7 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)

8 Pre-label the 14 pre-seeded plates (prepared in step 5-6) with the relevant strain name and date, i.e. 7 x plates labelled "N2" and 7 x plates labelled "*ret-1* LoF".

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

8.2 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.

8.3 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.

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

8.5 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)

9 In the morning, remove 24 square well 96-well plate from the cold room (prepared in steps 3-4) and weigh three random plates without their lids

Note: Number of plates will depend on the experiment being conducted

10 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).

11 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 ret-1_02_rep8, where:
- 'ret-1' is the strain to be tested
- '_02' is the unique drug source plate number
- '_rep8' is the imaging run number that day

12 Remove drug source plates (made in section 2) from the -20 °C freezer and allow to thaw at room temperature.

13 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.

14 Pre-label skirted 96-well PCR plates with drug source plate information (e.g. ret-1_01) that will be used to make up the diluted drug plates.

15 Next to a flame, dispense 9 µL of sterile water into each well of the pre-labeled dilution plates (step 14) using multichannel pipette and reagent reservoir.

16 Using VIAFLO multi-well dispenser on the 'Tom_Stock_Dilution' custom program, premix drug in the drug source plates [position A of VIAFLO], and then transfer 1 µL drug in DMSO to the diluted

drug plates prefilled with water (step 15) [position B of VIAFLO].

This creates a 1:10 dilution of the master compound stock, i.e. 100 mM -> 10 mM drug in diluted source plate

17 Repeat for all drug stock plates.

18 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 10).

19 Use VIAFLO on the 'Tom_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. 10 mM diluted drug stock -> 100 µM final imaging plate concentration:

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

20 Repeat steps 18-19 until all tracking plates have been dosed with the relevant compounds.

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

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

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

overnight.

Tracking

24 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.

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

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

27 Repeat steps 25-26.

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

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

Protocol

NAME

COPAS wormsorter

CREATED BY

Ida Barlow

PREVIEW

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

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

32 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

33 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

34 Transfer videos to BehavGenom and analyse with Tierpsy.