Mar 01, 2022

Response of Disease Model Mutants to Cholinergic Drugs

Thomas J. O'Brien[1]

[1]Imperial College London

dx.doi.org/10.17504/protocols.io.b5p7q5rn

Behavioural Genomics

Thomas J. O'Brien
Imperial College London

Protocol for testing if disease model mutant *C. elegans* strains show increased resistance/sensitivity to the action of cholinergic drugs after 1 and 4 hour exposure times.

DOI

dx.doi.org/10.17504/protocols.io.b5p7q5rn

protocol ,

Feb 28, 2022

Mar 01, 2022

58847

For the 1 hour time point it is important to get plates into the incubator with the lids off as quickly as possible to allow the wells to dry out prior to imaging.

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

1 Pick 10 x L4 worms onto 4 x 90mm NGM-agar plates pre-seeded with *E. coli* OP50 for each strain (remember to include a relevant control strain).

e.g. if looking to compare the response of one mutant to N2 reference: you would have 4 x 90

mm plates with strain 1 and 4 x 90 mm plates with N2

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

2 Prepare 1 x 96-well source plate per compound to be tested

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

e.g. here compounds were tested at a maximum concentration of 100 µM, therefore make a 0.1 M top-stock

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 3 x concentrations of drugs (in this case 0.1, 0.01 and 0.001 M)

2.4 Dispense 30 µL of the drug stocks into the relevant wells of a 96-well round bottomed micro-titre plate. Remember to include control wells (i.e. the drug solvent only) and label the plates with a frost-proof marker pen.

For this experiment I dispensed each drug concentration into 2 rows of a plate. Allowing 12 well replicates for the control and 12 well replicates for the mutant strain per compound, per concentration. This layout also allowed me 2 x rows for the solvent only controls.

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

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

3 Prepare 1 L no peptone NGM-agar and autoclave

Citation: Thomas J. O'Brien Response of Disease Model Mutants to Cholinergic Drugs
https://dx.doi.org/10.17504/protocols.io.b5p7q5rn

4 Once agar has cooled to around 65°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 in an airtight container at 4°C

(For this experiment 4 drugs were tested across three independent imaging days on two disease model mutant strains, totalling 24 tracking plates)

Dispensing agar into multiwell plates
by Saul Moore
PREVIEW RUN

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

5 Dry 2 x 150 mm NGM-agar plates per strain (including 2 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

Bleach synchronisation of C. elegans
by Ida Barlow
PREVIEW RUN

Refeed L1s (3 days prior to tracking)

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

8.1 Use a 3 mL plastic pasteur pipette to carefully remove the supernatant (leaving ~0.5 mL) then resuspend the worm pellet by gently flicking the tube

8.2 Using a clean rubber teat and glass pipette per strain, carefully aspirate the L1

Citation: Thomas J. O'Brien Response of Disease Model Mutants to Cholinergic Drugs
https://dx.doi.org/10.17504/protocols.io.b5p7q5rn

worm suspension and drop 4 small droplets around the edges of the pre-seeded 150 mm plates (prepared in step 6)

[This will yield 2 x 150 mm plates of refed L1s per strain being studied + controls]

8.3 Allow to grow at 20°C

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

9 In the morning, remove 1 square well 96-well plate from the cold room per strain and compound to be tested and weigh three random plates without their lids

(*Note*: If using the layout suggested above each plate will contain reference and control strains. Hence, if testing 2 x strains and 4 x compounds, remove 8 plates from the cold room)

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 aldicarb_hlb-1_01, where:
- 'aldicarb' is the drug to be tested
- 'hlb-1' is the strain of interest
- '01' is the imaging run number that day

12 Remove drug source plates (made in step 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 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. aldicarb_01) that will be used to make up the diluted drug plates

15 Next to a flame, dispense 7 µL of sterile water into each well of the pre-labeled dilution plates (step 14) using multichannel pipette and reagent reservoir

Citation: Thomas J. O'Brien Response of Disease Model Mutants to Cholinergic Drugs
https://dx.doi.org/10.17504/protocols.io.b5p7q5rn

16 Using VIAFLO multi-well dispenser on the 'BG_STOCK' custom program, premix drug in the drug source plates [position A of VIAFLO], and then transfer 0.5 µL drug in DMSO to the diluted drug plates prefilled with water (step 15) [position B of VIAFLO] -- Repeat for all drugs

17 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)

18 Use VIAFLO on the 'BG_AGAR' custom program to transfer 3 µL of diluted drug and water mixture onto the corresponding pre-labelled tracking plate [position A of VIAFLO]

19 Repeat steps 17-18 until all tracking plates have been dosed with the relevant compounds

20 Prepare 1:10 dilution of OP50 in M9 in a small, sterile bottle:
- 5 mL OP50
- 45 mL M9

21 Use the VIAFILL dispenser to seed all the tracking plates with 5 µL bacterial suspension per well

Tracking

22 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.

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

24 Carefully remove and discard the supernatent and refill with M9

25 Repeat steps 23-24

Citation: Thomas J. O'Brien Response of Disease Model Mutants to Cholinergic Drugs
https://dx.doi.org/10.17504/protocols.io.b5p7q5rn

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

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

COPAS wormsorter
by Ida Barlow
PREVIEW RUN

28 Allow liquid to dry off by placing imaging plates in 20°C incubator with lid off for 30 minutes

29 Expose worms to the drugs for 1 and 4 hours, as calculated from the middle wormsorter time and allow worms to acclimate for 30 minutes in the cave prior to imaging. Remember to return plates, agar-side up with lids on, to the 20°C incubator in between the 1 and 4 hour time points.

For example:
- wormsorter start time 10 :00
- wormsorter end time 10:40
- middle wormsorter time 10:20

1 hour exposure:
- cave time = 10:50
- imaging start time = 11: 20

4 hour exposure:
- cave time 11:50
- imaging start time 12:20

30 Image on the multi-camera tracker (Hydra) using 'run_syngenta_experiment_v2.py' protocol script (5 mins prestim; 6 mins bluelight with 60 sec OFF, [10sec ON, 90sec OFF] x 3 times; 5 mins postsim)

31 Transfer videos to BehavGenom and analyse with Tierpsy

Citation: Thomas J. O'Brien Response of Disease Model Mutants to Cholinergic Drugs
https://dx.doi.org/10.17504/protocols.io.b5p7q5rn