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Finding NHR mutants with higher sensitivity to nematocide drugs

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John Bergqvist[1], Julia Riedl[1]

[1]MRC Laboratory of Medical Sciences, Imperial College London

Behavioural Genomics

John Bergqvist
MRC Laboratory of Medical Sciences, Imperial College London

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We use this protocol and it's working

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Abstract

This protocol explores the sensitivity of *C. elegans* Nuclear Hormone Receptor (NHR) mutants to known nematocidial drugs using pixel variation as the data output for paralysed/killed worms.

Materials

For a screen of 24 NHR mutants and N2 control (25 strains):

- 50× 100 mm plates
- 25× 60 mm plates
- 75× 15 mL falcon tubes
- 4× 50 mL
- 15× 1.5 mL Eppendorf
- 14× 2 mL Eppendorf
- 25x Round, flat-bottom 96-well NUNCLON plates
- 1x V-bottom, deep-well 96-well plate
- 2× 500 mL Erlenmeyer flasks
- 25x plastic sterile loops

- Aldicarb (https://www.sigmaaldrich.com/GB/en/substance/aldicarb19026116063)
- Levamisole hydrochloride (https://www.sigmaaldrich.com/GB/en/product/sial/phr1798?srsltid=AfmBOoqaO8_QOygF3i0uh2vhOiWfLH963CbR8KRelVQG5z-JO6598xME)
- Ivermectin (https://www.sigmaaldrich.com/GB/en/product/sigma/i8898?srsltid=AfmBOoqdZ5pXKLH_hstYsfLxxI0uDFq1Tut8D1JjaT-hjpFVZQ6C8ZoN)
- Chlorpromazine hydrochloride (https://www.sigmaaldrich.com/GB/en/product/mm/215921?srsltid=AfmBOooT3uIvWf6HEaFCowDpdNMnnV32CZQHvH9ssnHeV9YONdGViBX5)

- M9
- S-medium
- DMSO
- Sterile water
- MilliQ water
- 16% Paraformaldehyde

Day -5.

1 **Making adult worm population**
Pick 20 worms onto a OP50-seeded 100 mm plate with NGM and incubate the worms at 25°C for ~24h.

2 **Making PFA-killed OP50**
Make an OP50 O/N culture by pipetting 20 µL of maintenance OP50 culture (OD = 1.0) into 1 L LB broth. Leave shaking at 200 rpm and 37°C O/N.

Day -4.

3 **Making adult worm population**
Remove the adults from the plate by picking. Incubate plates at 20°C until Day -1.

4 **Making PFA-killed OP50**
Make PFA-treated OP50 (OD = 2.0) out of the OP50 O/N culture using the protocol linked below.

Final resuspension of the pellets should be done with S-medium.

Protocol

NAME
PFA treatment of OP50

CREATED BY
Bonnie Evans

Preview

5 Make Stock 1 concentrations of Aldicarb, Levamisole and Chlorpromazine.

Stock 1 - Aldicarb (24 mM):
Weigh 10 mg in a 5 mL Eppendorf tube. Add 2190 µL of sterile water. Vortex extensively to dissolve.
Stock 1 - Levamisole (50 mM):
Weigh 10 mg in a 2 mL Eppendorf tube. Add 831 µL of sterile water. Vortex extensively to dissolve.
Stock 1 - Chlorpromazine (20 mM):

Weigh 10 mg in a 2 mL Eppendorf tube. Add 831 µL of sterile water. Vortex extensively to dissolve.

6 Make 1 mL Stock 2 concentrations of Aldicarb, Levamisole and Chlorpromazine in 1.5 mL Eppendorf tubes.

Stock 2 - Aldicarb (4.8 mM)
Add 200 µL of Stock 1 Aldicarb (24 mM) to 800 µL sterile water. Vortex.
Stock 2 - Levamisole (1.2 mM)
Add 24 µL of Stock 1 Levamisole (50 mM) to 976 µL sterile water. Vortex.
Stock 2 - Chlorpromazine (1.2 mM)
Add 60 µL of Stock 1 Chlorpromazine (20 mM) to 940 µL sterile water. Vortex.

7 Store Stock 1 and Stock 2 vials in -20°C.

Day -1.

8 Estimate the number of eggs on the plates in the morning.

- If there is a high number of eggs on the plate of adult worms, proceed from **Step 11**
- If there is not enough number of eggs on the plate of adult worms, proceed from **Step 9**

9 Wash adult worms off the 100 mm plate and transfer to a 15 mL Falcon tube. Wash once in M9.

10 Drop the adult worms onto a OP50-seeded 100 mm plates with NGM and incubate at 25°C for 4h.

11 Wash adults off the plates. Loosen the eggs from the plate using a plastic sterile loop and M9 and transfer to a 15 mL falcon tube. Wash twice in M9 and transfer the eggs to the 60 mm plate with M9.

12 Leave the 60 mm plate with eggs and M9 in the 20°C incubator O/N to synchronise.

Day 0.

13 Make Stock 1 concentrations of Ivermectin (always made fresh on the day of the experiment).

Stock 1 - Ivermectin (12 mM):
Weigh 10 mg in a 2 mL Eppendorf tube. Add 952 µL of DMSO. Vortex extensively to dissolve.

14 Prepare the drug-plate in a deep-well 96-well plate.

STEP CASE

N2 concentration determination 15 steps

These steps describe the steps done for finding the concentration range that should be applied for the whole screen

15 Plate layout of the final concentrations in 96 well plates to achive through the below steps (not drug-plate):

Concentration (µM)	1	2	3	4	5	6	7	8	9	10	11	12
A. Aldicarb	0.039	0.078	0.156	0.313	0.625	1.25	2.5	5	10	20	40	80
B. Levamisole	1	3	3.5	4	4.5	5	5.5	6	6.25	50	100	200
C. Ivermectin	0.008	0.010	0.015	0.030	0.060	0.070	0.080	0.090	0.100	0.120	0.240	0.480
D. Chlorpormazine	6.25	12.5	25	28	31	33	35	40	45	50	100	200
E. Sterile water	solvent control	solvent control	solvent control	solvent control	solvent control	solvent control	solvent control	solvent control	solvent control	solvent control	solvent control	solvent control
F. DMSO	solvent control	solvent control	solvent control	solvent control	solvent control	solvent control	solvent control	solvent control	solvent control	solvent control	solvent control	solvent control

16 Make the working concentrations for Ivermectin in individual 1.5 mL Eppendorf tubes for each 12 wells with different concentrations according to the table below (colour-coded according to the concentrations in the wells and which concentration to use to make a new, lower concentration).

Stock 2 Ivermectin (96 µM):
Add 4 µL of Stock 1 Ivermectin (12 mM) to 496 µL DMSO. Vortex.

well number	Concentration	Stock 2 Ivermectin (96 µM)
C12	96 µM	straight from stock
C11	48 µM	200 µL (96 µM) + 200 µL DMSO
C10	24 µM	50 µL (96 µM) + 150 µL DMSO
C9	20 µM	42 µL (96 µM) + 158 µL DMSO
C8	18 µM	38 µL (96 µM) + 162 µL DMSO
C7	16 µM	67 µL (48 µM) + 133 µL DMSO
C6	14 µM	58 µL (48 µM) + 142 µL DMSO
C5	12 µM	100 µL (48 µM) + 300 µL DMSO
C4	6 µM	200 µL (12 µM) + 200 µL DMSO
C3	3 µM	200 µL (6 µM) + 200 µL DMSO
C2	2 µM	200 µL (3 µM) + 100 µL DMSO
C1	1.6 µM	80 µL (2 µM) + 20 µL DMSO

17 **Adding Ivermectin to drug-plate:**

1. In **row C**, add 3 µL of the Ivermectin concentrations into their respective wells according to the table above.
2. Add 97 µL of sterile water to each of the wells in row C. Mix thoroughly by pipetting up and down ~30 times.

18 **Adding Aldicarb to drug-plate:**

1. In **row A**, add the volumes of sterile water according to the table below first (colour-coded according to the concentrations in the wells and which concentration to use to make a new, lower concentration).
2. Next, add Aldicarb according to the table below and mix thoroughly by pipetting up and down ~30 times.

well number	Concentration	Stock 2 Aldicarb (4.8 mM)
A12	480 µM	20 µL (4.8 mM) + 180 µL S.H2O
A11	240 µM	100 µL (480 µM) + 100 µL S.H2O
A10	120 µM	100 µL (240 µM) + 100 µL S.H2O
A9	60 µM	100 µL (120 µM) + 100 µL S.H2O
A8	30 µM	100 µL (60 µM) + 100 µL S.H2O
A7	15 µM	100 µL (30 µM) + 100 µL S.H2O
A6	7.5 µM	100 µL (15 µM) + 100 µL S.H2O
A5	3.75 µM	100 µL (7.5 µM) + 100 µL S.H2O
A4	1.875 µM	100 µL (3.75 µM) + 100 µL S.H2O
A3	0.938 µM	100 µL (1.875 µM) + 100 µL S.H2O
A2	0.469 µM	100 µL (0.938 µM) + 100 µL S.H2O
A1	0.234 µM	100 µL (0.469 µM) + 100 µL S.H2O

19 **Adding Levamisole to drug-plate:**

1. In **row B**, add the volumes of sterile water according to the table below first (colour-coded according to the concentrations in the wells and which concentration to use to make a new, lower concentration).
2. Next, add Levamisole according to the table below and mix thoroughly by pipetting up and down ~30 times.

well number	Concentration	Stock 2 Levamisole (1.2 mM)
B12	1.2 mM	straight from stock (1.2 mM)
B11	600 µM	200 µL (1.2 mM) + 200 µL S.H2O
B10	300 µM	100 µL (600 µM) + 100 µL S.H2O
B9	37.5 µM	25 µL (300 µM) + 175 µL S.H2O
B8	36 µM	12 µL (300 µM) + 88 µL S.H2O
B7	33 µM	11 µL (300 µM) + 89 µL S.H2O
B6	30 µM	10 µL (300 µM) + 90 µL S.H2O
B5	27 µM	9 µL (300 µM) + 91 µL S.H2O
B4	24 µM	8 µL (300 µM) + 92 µL S.H2O
B3	21 µM	7 µL (300 µM) + 93 µL S.H2O
B2	18 µM	6 µL (300 µM) + 94 µL S.H2O
B1	6 µM	67 µL (18 µM) + 133 µL S.H2O

20 **Adding Chlorpromazine to drug-plate:**

1. In **row D**, add the volumes of sterile water according to the table below first (colour-coded according to the concentrations in the wells and which concentration to use to make a new, lower concentration).
2. Next, add Chlorpromazine according to the table below and mix thoroughly by pipetting up and down ~30 times.

well number	Concentration	Stock 2 Chlorpromazine (1.2 mM)
D12	1.2 mM	straight from stock
D11	600 µM	250 µL (1.2 mM) + 250 µL S.H2O
D10	300 µM	50 µL (600 µM) + 50 µL S.H2O
D9	270 µM	45 µL (600 µM) + 55 µL S.H2O
D8	240 µM	40 µL (600 µM) + 60 µL S.H2O
D7	210 µM	35 µL (600 µM) + 65 µL S.H2O
D6	198 µM	33 µL (600 µM) + 67 µL S.H2O
D5	186 µM	31 µL (600 µM) + 69 µL S.H2O
D4	168 µM	28 µL (600 µM) + 72 µL S.H2O
D3	150 µM	50 µL (600 µM) + 150 µL S.H2O
D2	75 µM	100 µL (150 µM) + 100 µL S.H2O
D1	37.5 µM	100 µL (75 µM) + 100 µL S.H2O

21 **Adding solvent negative controls to drug-plate:**

1. In **row E**, add 100 µL of sterile water to each of the wells.
2. In **row F**, add 3 µL of DMSO to each of the wells. Next, add 97 µL of sterile water and mix thouroughly by pipetting up and down ~30 times.

22 Using the ViaFlow, add 10 µL of the drugs onto 7 flat and round Nunclon 96-well plates.

23 Using the ViaFill with the small tubing, prime the tubing. Then dispense 30 µL of the PFA-killed OP50 (OD=2.0) to all plates. Wash the tubing with MilliQ water.

24 Spin down the now synchronised L1s at Program 1 and wash with fresh M9 once.

25 Adjust the volume of M9 to acquire ~20 worms per 20 µL by pipetting 20 µL onto a glass slide or spare plate lid with a 200 µL pipette tip.

26 Add a magnetic stirrer bar to the glass bottle with the worms and place the bottle on the magnetic stirrer.

Change the volume of the ViaFill programme to 20 µL.

Prime the small tubing with the worms. As soon as liquid is clearly coming out of all 8 tubes, start dispensing worms into 96-well plates.

27 Check all wells to ensure worms were dispensed in all wells.

28 Incubate the plates at 20°C for 72h (Day 3)

Day 3.

29 After 72h since adding the worms to the plates, record the plates 4 wells at a time according to the specifications below using the Basler camera attached to a light microscope and the Basler software 'pylon Viewer' (6.2.4.9387).

Folder structure:

Save images in the correct folder:

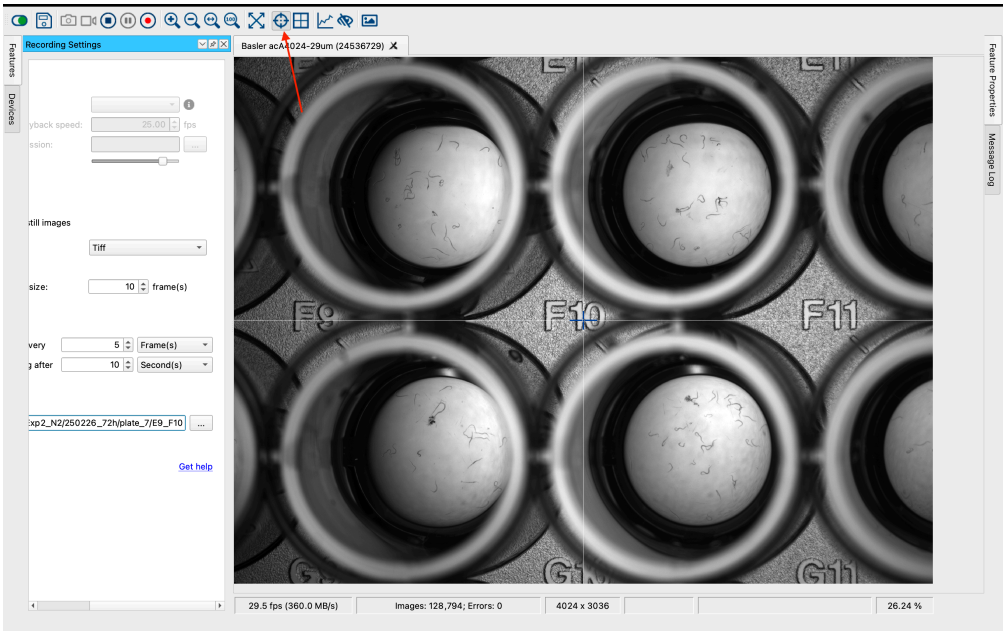
- Ensure you have 400 GB free space on your computer.
- Copy the filepath to the folder on your local computer (e.g. /Users/jb3623/Desktop/basler/video/Exp2_N2/250226_72h/plate_2/A1_B2)
- For each new recording, change the subdirectory name from e.g. 'A1_B2' to 'A3_B4'.

Plate positioning:

It is essential that the plate is oriented according to the example below (starting with A1_B2).

Click on the 'scope' symbol indicated by the red arrow to activate the grid.

- Horizontal line crosses the sections where the top and bottom wells meet on both the left and the right side.
- Vetical line crosses the two wells on the right hand side just before the dark rings.



Recording Settings:

- Output format: Tiff
- Recording buffer size: 10 frame(s)
- Record a frame every: 5 Frame(s)
- Stop recording after: 10 seconds