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Syngenta drug screen

Forked from Drug tracking on hydra

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ABSTRACT

Protocol for preparing worms, preparing drug plates, dispensing worms with wormsorter and tracking on Hydra rigs

MATERIALS

NAME	CATALOG #	VENDOR
Whatman Square Well 96 well plates	WHAT7701-1651	VWR Scientific

Pick L4 worms for bleaching (-9 days) 30m

1 Pick L4 worms onto 4 x OP50-seeded NGM 90mm plates (10 worms per plate) for day 1 of tracking

Pick L4 worms for bleaching and pour 96WPs (- 8 days) 2h

2 Pick L4 worms onto 4 x OP50-seeded NGM 90mm plates (10 worms per plate) for day 2 of tracking

3 Prepare 1.5L low peptone NGM and autoclave

4 Once agar has cooled and salts have been added pour agar into 65 x 96 well plates using VIAFILL dispenser. Dispense **200 µl** per well. Once cooled, store lidside down in airtight container at **4 °C** .

Bleach worms (- 5 days) 1h 30m

5 Bleach worms prepared for day 1 tracking: 60h

Bleach synchronisation of C. elegans
by Ida Barlow
PREVIEW RUN

Adaptations:
- remix pellet after each wash

5.1 Wash hermaphrodites off plate with several ml of M9 solution and transfer to 15ml falcon tube (Fisher Scientific-Falcon 352096)

5.2 Fill falcon tube up to 15ml with M9 solution

5.3 Centrifuge for 2 minutes at 1500 rpm (RCF:210, ascending 9; descending 7) – program 1

Program 1 retains the worms as pellets and the bacteria is suspended as the supernatant

The descending is slow as the worm pellet is lose at this stage which we don't want to break

5.4 Remove supernatant using a plastic Pasteur pipette taking care not to disturb pellet
Leave atleast 0.5ml M9 to avoid disturbing the pellet

5.5 Fill the tube with M9 upto 15ml

5.6 Spin program 1

5.7 *Repeat steps 4-6*

5.8 On final wash remove as much supernatant as possible and add M9 upto 4ml

5.9 Add 4ml 2X Bleach solution (From here onwards try to work as quickly as possible to avoid over-exposure of the worms to the bleach)

USE FRESHLY PREPARED BLEACH EVERYTIME

2X Bleach solution:

5% Sodium hypochlorite solution - 4ml
Sterile water - 3.5 ml
1M NaOH solution - 2.5 ml
TOTAL - 10 ml

5.10 Vortex on maximum setting for 4 min (no more as this will damage the eggs)

Makesure the vortex forms

After vortexing, top up the tube with M9 till 15ml

5.11 Centrifuge for 2 mins at 2500rpm (RCF:590, ascending 9; descending 7) – program 2

(Always check the program on the centrifuge before using it)

5.12 Remove supernatant by pouring into waste bottle – pellet should be compact and yellow in colour at bottom of falcon, but be careful not to lose

5.13 Add 15ml M9

5.14 Centrifuge at program 2

5.15 Repeat steps 12-14 four more times

The number of washes is crucial here as we need to get rid of all the bleach

5.16 After final wash add 15ml M9 and store eggs/larvae in the falcon on the rotator that is constantly spinning at 20^{o}C, until feeding

L1 arrested larvae can be starved for up to 5 days before refeeding

5.17 Centrifuge larvae on program 2 to pellet

5.18 Remove supernatant with plastic Pasteur pipette

The pellet is lose here so makesure not to disturb it

5.19 Add 15ml M9, spin to wash

5.20 On final wash leave 0.5ml M9 in falcon

5.21 Resuspend the pellet by gently tapping the tube/flicking it

5.22 Place droplet containing larvae onto seeded plate and allow to grow to desired developmental state (ie. 2 days for L4s, 2.5 days for young adults)

Use glass pipette to place the droplet onto seeded plate, avoid using plastic pipette as larvae will stick to it

Development times at 20°C:

- 2 days for L4s
- 2.5 days for young adults

Note:

- If you feed larvae within 12hrs of bleaching then they develop faster than the longer arrested ones
- It is a good practice to bleach in two tubes in parallel
- If you drop the tube at any point of the process, makesure to transfer the contents into a new tube as the dropped tube may get cracked resulting in loss of worms during centrifugation/vortexing
- Any unused larvae can be topped up with M9 and stored spinning in the rotator to be re-used
- Use clean autoclaved rubber bulbs for the refeeding everytime to avoid contamination
- Put the used bulb in the box labelled 'Used Teets'

Stages	Grown at 20 C from L1	Grown at 25 C from L1
L1 division	11.7hrs	9hrs
Mid L1	16.9hrs	13hrs
First L2 division	22.1hrs	17hrs
Between L2 divisions	23.4hrs	18hrs
Second L2 divisions	24.3hrs	19hrs
Mid L2	29.9hrs	23hrs
L3 division	32.5hrs	25hrs
Mid L3	37.7hrs	29hrs
L4 division	42.9hrs	33hrs
Mid L4	49.4hrs	38hrs
Early adult	55.9hrs	43hrs
Adult	62.4hrs	48hrs

Table of Development times for different temperatures

Bleach worms (- 4 days) 1h 30m

6 Bleach worms prepared for day 2 tracking and keep at **20 °C** in rotatotor until refeeding:

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Refeed L1s (- 3 days) 1h 30m

7 At 17:00

Refeed L1s for day 1 tracking onto 5 x 150mm plates as in protocol above by dropping 4 small droplets around the edges of the plate (off food).

Wash worms once before refeeding

Keep worms at **20 °C**

Refeed L1s (- 2 days) 1h 30m

8 At 17:00

Refeed L1s for day 2 tracking onto 5 x 150mm plates as in protocol above by dropping 4 small droplets around the edges of the plate (off food).

Wash worms once before refeeding

Keep worms at **20 °C**

Dry 96 well plates (-2 days) 3h

9 Weigh 3 random plates

10 Place in flow hood for 3 hours to allow to dry (with lids off)

11 Weigh 3 random plates

Prepare drugs onto plates (-1 days) 3h

12 Use VIAFLO to fill 30 x SOURCE plates with **7 µl** water and **0.5 µl** drug from STOCK plates.

13 Label each DESTINATION plate with the source plate the DESTINATION SLOT in the opentrons robot.

14 Prepare drug source plates in skirted 96 well plate so that each column corresponds to a single drug at a single concentration. The final concentration of DMSO should be 0.1%.

15 Run opentrons robot using prepared protocol.

NB.
- One drug source plate with each column corresponding to a drug for use with a multichannel pipette.
- Destination and source plate locations are specified.
- The opentrons robot will randomise the column locations from each source plate to a destination plate
- The date is used as the random seed

15.1 Protocol parameters:

```
# multichannel pipette parameters and tipracks
multi_pipette_type = 'p10-Multi'
multi_pipette_mount = 'left'

tiprackdrugs_slots = ['3']
tiprackdrugs_type = 'opentrons-tiprack-10ul'
tiprackdrugs_startfrom = '1'

tiprackH2O_slot = '6'
tiprackH2O_type = 'opentrons-tiprack-10ul'
# tiprackH2O_startfrom = '1'

# water trough
H2O_source_slot = '9'
H2O_source_type = 'trough-12row'
H2O_source_well = 'A1'
H2O_volume = 5

# drugs source
drugs_source_slots = '2'#,'5','8','11']
drugs_source_type = '96-well-plate-pcr-thermofisher'
frombottom_off = +0.3 # mm from bottom of src wells
drugs_volume = 3

# destination plates
agar_thickness = +3.7 # mm from the bottom of the well for 200ul agar per well
destination_slots = ['1','4','7', '5']
destination_type = '96-well-plate-sqfb-whatman'

n_columns = 12

# create mapping from sources to destination.
seed = 20191031 # for reproducibility. Let's use the experimental date for the actual experiment, something else for debugging
np.random.seed(seed)
```

15.2 Make sure all labware is loaded correctly

15.3 Run protocol and monitor robot to ensure all the tips are removed

15.4 Tip racks have to replaced after each destination plate is filled

16 Allow the plates to dry for 30 minutes

Seed plates (-1 days)

17 Use VIAFILL dispenser to dispense **5 µl** 1:10 diluted OP50 into each well of each drug plate

18 Allow to dry for 30 minutes under the flow hood

19 Keep at room temperature over night (covered in the dark as some drugs are light-sensitive)

Disepensing worms

20 Wash worms off the 150mm plates with M9 using a pasteur pipette into 15ml falcons

21 Spin at **1500 rpm , ascending 9, descending 7** for 2 minutes to pellet the worms

22 Remove supernatent and fill M9

23 Repeats steps 12-13 two more times

24 After final wash fill falcon with M9, transfer contents of 15mL falcons to 50mL and fill up to 45mL with M9.

25 Use wormsorter to dispense 2 worms per well

26 Allow liquid to dry off the plates for 30 mins under the flow hood

27 Incubate in drug for 4 hours

28 Hydra tracking : 15 mins 25fps, exposure 25000msec