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Disease model screen protocol

Forked from Disease model screen protocol

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1 Works for me dx.doi.org/10.17504/protocols.io.bsicncaw

Behavioural Genomics

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SUBMIT TO PLOS ONE

ABSTRACT

Protocol for screening the disease model *C. elegans* strains under baseline and bluelight conditions using the Hydra (Loopbio) imaging rigs. This is the protocol for one day of imaging and should be repeated at least three times to get replicates from three separate days. The number of imaging runs depends on the number of strains being screened and whether strains are sorted into 96 well plates row-wise (7 strains per plate) or column-wise (11 strains per plate).

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PROTOCOL CITATION

Ida Barlow 2021. Disease model screen protocol. **protocols.io** https://dx.doi.org/10.17504/protocols.io.bsicncaw

FORK NOTE

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GUIDELINES

Careful planning of how drugs to be arranged in plates and the number of strains is required before undertaking screening experiments. Using a google calendar to pre-plan timings and days is advised in order to efficiently manage the workload

MATERIALS TEXT

VIAFLO
96 channel pipette
Integra **VIAFLO 96**

VIAFILL
reagent dispenser
Integra **VIAFILL**

BEFORE STARTING

96 well plates containing the drug library at all doses need to be randomised by column using the OpenTrons robot to create 3 stock plates for each library plate.

Pick L4 worms for bleaching (-10 days before tracking; eg Monday) 1h

1 Pick 5 x L4s per strain onto 3 x 60mm plates (pre-seeded with OP50) for each strain

2 Take out 90mm plates from cold room to allow to dry at room temperature

3 x 90mm plates per strain being imaged

Pour 96WPs (anytime up to 2 days before tracking) 4h

3 Prepare appropriate volume of low peptone NGM and autoclave

How to calculate volume of agar required

number of imaging runs = (number of strains) / fill_rows (7) or fill_columns (11)
number of plates = number of imaging runs * number of rigs (default=5 hydras)

volume agar (mL) = number of plates * 96 * 0.2

Round up the volume of agar required to the nearest half litre

NB.
fill_rows if filling plates with each row as a different strain
fill_columns if filling plates with each column as a different strain

every plate must have a row/column saved for controls, so fill_rows/columns = number_columns/rows - 1

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4 Once agar has cooled to around **65 °C** , add the salts and dispense agar into 96 well plates using VIAFILL dispenser. Dispense **200 µl** per well. Once cooled, store agar side up with one lid per stack of 5 plates in an airtight container at **4 °C**

Seed plates, bleach worms (-5 days before tracking eg Friday) 4h

5 Seed with OP50 the 90mm plates that have been drying at room temperature

6 Bleach worms prepared for day 1 of tracking

Bleach synchronisation of C. elegans
by Ida Barlow
PREVIEW RUN

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

6.2 Fill falcon tube up to 15ml with M9 solution

6.3 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

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

6.5 Fill the tube with M9 upto 15ml

6.6 Spin program 1

6.7 *Repeat steps 4-6*

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

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6.9 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

6.10 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

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

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

6.12 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

6.13 Add 15ml M9

6.14 Centrifuge at program 2

6.15 Repeat steps 12-14 four more times

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

6.16 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

6.17 Centrifuge larvae on program 2 to pellet

Remove supernatant with plastic Pasteur pipette

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6.18 The pellet is lose here so makesure not to disturb it

6.19 Add 15ml M9, spin to wash

6.20 On final wash leave 0.5ml M9 in falcon

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

6.22 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

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Refeed L1 (-3 days before tracking eg Sunday) 4h

7 At 11:00 spin L1s at 2500rpm. Remove supernatent and using glass pipette, drop 3-4 small droplets around the edges of the plate (off food) onto 3 x seeded 90mm plates per strain.

Allow to grow at **20 °C**

Dry and seed 96 well plates (-1 days before tracking eg. Tuesday) 3h

8 Take required number of 96 well plates from the cold room, and weigh three random plates without their lids

9 Place in cabinet dryer (setting 2) and allow to dry for 2-3 hours with lids off

10 Weigh 3 random plates and verify that at least 3-5% reduction in weight

11 Prepare 1:10 dilution of OP50 in M9 in small bottle

20ml total volume is sufficient for 10 96 well plates

12 Using VIAFILL dispenser, seed all the imaging plates with **5 µl** per well

13 Leave plates overnight at room temperature with lid

Imaging (eg Wednesday)

14 Pre-label imaging plates (square well) with IR visible pen or labels

Use naming system:
SXX_RYY

XX = imaging run number
YY = rig (hydra number)

15 Wash one batch of worms (strains for imminent imaging run) off 90mm paltes with M9 buffer using pasteur pipette into 15ml falcons

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16 Spin at 1500rpm for 2 minutes to pellet the worms

17 Remove supernatent and fill with M9

18 Repeat steps 18-19

19 After final wash, fill falcon with M9 and transfer contents from 15ml falcon to a 50ml and fill up 30ml with M9

20 Record start time for sorting this batch of worms

21 Use COPAS wormsorter to dispense 3 worms per well (pure, no double) into specified wells for 5 x 96WPs. Use pre-made YYYYMMDD_wormsorter.csv to plan and determine which strains to dispense into each row of each plate

22 Between each strain, wash with purified H_2O by placing water falcon into sample cup and washing through by selecting Maintenance -> FlushSample

23 Once the plates for this imaging run have been filled, record the end time

24 Allow liquid to dry off by placing imaging plates in **20 °C** incubator with lid off for 30 minutes, then replace lids and keep in 20oC incubator

25 Repeat steps 17-25 for the next batch of worms for the next imaging run

26 For each imaging run, allow 30 minutes drying, 30 minutes in 20oC incubator with lids on and then 30 minutes acclimation in worm cave before imaging on hydra (calculate times from middle of wormsorter time)

27 Imaging on hydra using protocol script (5 mins prestim; 6 mins bluelight with 60 sec OFF, [10sec ON, 90sec OFF] x 3 times; 5 mins postsim)

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~/scripts$ **python run_syngenta_experiment_v2.py -f disease_models_run1 – r 01 02 03 04 05**

-f : project/file name for batch of recordings with run number at the end:
! do not use blank spaces in file name!
! add run number for each run of tracking on each day !

-r : specifies which rigs to start recording on

Transfer files from NAS

28 Enter project/file name pattern to search for (eg disease_models; nb. all lower case)

29 Enter the folder where the files should be transferred

A new folder is automatically generated for each day of imaging

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