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Disease model screen protocol v2

Forked from Disease model screen protocol

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Behavioural Genomics

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

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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), or if each strain has its own plate (1 strain per plate). N2s should always be screened on the same day as a comparison.

Guidelines

Careful planning of how drugs are 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

Equipment	
VIAFLO	NAME
96 channel pipette	TYPE
Integra	BRAND
VIAFLO 96	SKU
https://www.integra-biosciences.com/united-kingdom/en/electronic-pipettes/viaflo-96-viaflo-384#tech-info	LINK

Equipment	
VIAFILL	NAME
reagent dispenser	TYPE
Integra	BRAND
VIAFILL	SKU
https://www.integra-biosciences.com/united-kingdom/en/microplate-dispenser/viafill	LINK

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

1 Pick 5 x L4s per strain onto 3 × 60mm plates (pre-seeded with OP50) for each strain. For slow-growing strains, pick 10 adult hermaphrodites.

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

Note

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

Note

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

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 (5-6 days before tracking e.g. Thursday or Friday) 4h

5 **For slow-growing strains,** bleach one day before strains with normal growth (6 days before tracking)
For normal strains, bleach 5 days before tracking

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

7 Bleach worms prepared for day 1 of tracking

Protocol

NAME

Bleach synchronisation of C. elegans

CREATED BY

Ida Barlow

Preview

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

7.2 Fill falcon tube up to 15ml with M9 solution

7.3 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

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

7.5 Fill the tube with M9 upto 15ml

7.6 Spin program 1

7.7 *Repeat steps 4-6*

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

7.9 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

Note

2X Bleach solution:

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

7.10 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

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

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

7.12 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

7.13 Add 15ml M9

7.14 Centrifuge at program 2

7.15 Repeat steps 12-14 four more times

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

7.16 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

Note

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

7.17 Centrifuge larvae on program 2 to pellet

7.18 Remove supernatant with plastic Pasteur pipette

The pellet is lose here so makesure not to disturb it

7.19 Add 15ml M9, spin to wash

7.20 On final wash leave 0.5ml M9 in falcon

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

7.22 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

Note

Development times at 20^{o}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

Refeed L1 (3-4 days before tracking e.g. Saturday or Sunday)

4h

8 **For slow-growing strains,** refeed four (4) days before tracking

For normal strains, refeed three (3) days before tracking

9 At 17:00 spin L1s at 1500rpm. Remove supernatant 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 e.g. Tuesday)

3h

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

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

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

13 Using VIAFILL dispenser, seed all the imaging plates with 10 µL of PFA-killed OP50 bacteria per well

Protocol

NAME

PFA treatment of OP50

CREATED BY

Bonnie Evans

Preview

14 Let the bacteria dry, then leave plates agar-side up overnight at room temperature with lid

Imaging (e.g. Wednesday)

15 Create a new entry per plate in Airtable under the "Plates" tab. This should generate a unique plate ID for each tracking plate. Pre-label dried tracking plates with the strain name and Airtable plate ID, so that every plate on a single day of imaging has a unique

label. For example: N2 plate_895. Upload the corresponding metadata excel sheet to the "Well Information" column in Airtable → Plates.

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

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

18 Remove supernatent and fill with M9

19 Repeat steps 17-18

20 After final wash, fill each 15 mL falcon with M9. Transfer contents to a clean 100mL glass lab bottle labelled with the strain name on the side

21 Mix/swirl the worm solution and place two drops of 100 uL of worm suspension on an empty petri dish or other glass surface. Estimate the number of worms swimming in each 100 uL drop and divide by 10 to get the worm density per 10 uL. If too concentrated, add an appropriate amount of M9. If too diluted, spin again (program 1), remove M9, and re-count worm suspension

22 Use the VIAFILL to dispense 10 uL of worm solution per well of the pre-dried, seeded, 96-well imaging plate using the large cassette. Note the start time of dispensing

23 Between each strain, wash the VIAFILL tubing with purified H_2O by "priming" with water

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

25 Allow liquid to dry off by placing imaging plates in the microbial safety cabinet with lid off for 60 minutes, then use a microscope to check that wells are dry and keep, agar-side up, in the microbial safety cabinet.

26 Repeat steps 16-25 for the next batch of worms for the next imaging run

27 For each imaging run, allow 60 minutes drying and then 30 minutes acclimation in worm cave before imaging on hydra (calculate times from middle of dispensing time)

28 Use Airtable → Runs to image on the multi-camera tracker (Hydra) using 'run_syngenta_experiment_v2.py' (script 15) 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

Note: each plate should be one "run" in Airtable → Runs

29 Transfer videos to BehavGenom and analyse with Tierpsy