Oct 28, 2020

Syngenta divergent strain screen protocol

Ida Barlow[1], Adam Mcdermott-Rouse[1], Luigi Feriani[1]

[1]Imperial College London

1 Works for me dx.doi.org/10.17504/protocols.io.bn5zmg76

Behavioural Genomics

Ida Barlow

ABSTRACT

Protocol for screening the 12 divergent *C. elegans* strains with 100 Syngenta pesticide drugs at 3 concentrations and imaging under baseline and bluelight conditions using the Hydra (Loopbio) imaging rigs. The twelve strains were imaged over two days of tracking and on each day of tracking six strains were imaged across all drugs and all concentrations with 3 replicates for all conditions. This protocol should be repeated at least 3 times.

DOI

dx.doi.org/10.17504/protocols.io.bn5zmg76

PROTOCOL CITATION

Ida Barlow, Adam Mcdermott-Rouse, Luigi Feriani 2020. Syngenta divergent strain screen protocol. **protocols.io** https://dx.doi.org/10.17504/protocols.io.bn5zmg76

CREATED

Oct 28, 2020

LAST MODIFIED

Oct 28, 2020

PROTOCOL INTEGER ID

43929

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

ABSTRACT

Protocol for screening the 12 divergent *C. elegans* strains with 100 Syngenta pesticide drugs at 3 concentrations and imaging under baseline and bluelight conditions using the Hydra (Loopbio) imaging rigs. The twelve strains were imaged over two days of tracking and on each day of tracking six strains were imaged across all drugs and all concentrations with 3 replicates for all conditions. This protocol should be repeated at least 3 times.

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 (-9 days from first day of tracking) 1h

1 Pick 10 x L4s for 6 strains onto 3 x 90mm plates (pre-seeded with OP50) for each strain

Pick L4 worms for bleaching and pour 96WPs (-7 days) 4h

2 Pick 10 x L4s for 6 strains onto 3 x 90mm plates (pre-seeded with OP50) for each strain

3 Prepare 2 x 1.5L low peptone NGM and autoclave

4 Once agar has cooled to around **65 °C** , add the salts and dispense agar into 140 x square well 96 well plates using VIAFILL dispenser. Dispense **200 µl** per well. Once cooled, store agar side up in an airtight container at **4 °C**

Dry plates, bleach worms (-5 days) 4h

5 Dry 20 x 150mm plates in cabinet dryer (setting 2) or 3 hours

6 Seed 20 x 150mm plates with OP50 and leave to dry overnight at room temperature

7 Bleach worms prepared for day 1 of tracking

Bleach synchronisation of C. elegans
by Ida Barlow
PREVIEW RUN

Citation: Ida Barlow, Adam Mcdermott-Rouse, Luigi Feriani (10/28/2020). Syngenta divergent strain screen protocol. https://dx.doi.org/10.17504/protocols.io.bn5zmg76

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

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

Citation: Ida Barlow, Adam Mcdermott-Rouse, Luigi Feriani (10/28/2020). Syngenta divergent strain screen protocol. https://dx.doi.org/10.17504/protocols.io.bn5zmg76

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

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

Citation: Ida Barlow, Adam Mcdermott-Rouse, Luigi Feriani (10/28/2020). Syngenta divergent strain screen protocol. https://dx.doi.org/10.17504/protocols.io.bn5zmg76

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

Dry plates, bleach worms and refeed L1 (-3 days) 4h

8 Dry 20 x 150mm plates in cabinet dryer (setting 2) or 3 hours

9 Seed 20 x 150mm plates with OP50 and leave to dry overnight at room temperature

10 Bleach worms prepared for day 2 of tracking

Bleach synchronisation of C. elegans
by Ida Barlow

PREVIEW RUN

Citation: Ida Barlow, Adam Mcdermott-Rouse, Luigi Feriani (10/28/2020). Syngenta divergent strain screen protocol. https://dx.doi.org/10.17504/protocols.io.bn5zmg76

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

10.2 Fill falcon tube up to 15ml with M9 solution

10.3 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

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

10.5 Fill the tube with M9 upto 15ml

10.6 Spin program 1

10.7 *Repeat steps 4-6*

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

10.9 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

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

Makesure the vortex forms

Citation: Ida Barlow, Adam Mcdermott-Rouse, Luigi Feriani (10/28/2020). Syngenta divergent strain screen protocol. https://dx.doi.org/10.17504/protocols.io.bn5zmg76

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

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

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

10.12 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

10.13 Add 15ml M9

10.14 Centrifuge at program 2

10.15 Repeat steps 12-14 four more times

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

10.16 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

10.17 Centrifuge larvae on program 2 to pellet

10.18 Remove supernatant with plastic Pasteur pipette

The pellet is lose here so makesure not to disturb it

10.19 Add 15ml M9, spin to wash

10.20 On final wash leave 0.5ml M9 in falcon

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

10.22 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)

Citation: Ida Barlow, Adam Mcdermott-Rouse, Luigi Feriani (10/28/2020). Syngenta divergent strain screen protocol. https://dx.doi.org/10.17504/protocols.io.bn5zmg76

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

11 At 17:00, spin L1s for day 1 of tracking at 2500rpm. Remove supernatent and using glass pipette, drop 4 small droplets around the edges of the plate (off food) onto 3 x 150mm plates per strain.

Allow to grow at **20 °C**

Dry 96 well plates (-2 days) 3h

12 Take 65-70 poured 96 well plates from the cold room, and weigh three random plates without their lids

13 Place in cabinet dryer (setting 1.5-2) and allow to dry for 2-3 hours with lids off

Citation: Ida Barlow, Adam Mcdermott-Rouse, Luigi Feriani (10/28/2020). Syngenta divergent strain screen protocol. https://dx.doi.org/10.17504/protocols.io.bn5zmg76

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

15 leave overnight at room temperature with lid

Dispense drugs onto imaging plates using VIAFLO (-1 days from day 1 of tracking) 4h

16 Pre-label imaging plates (square well) with the imaging run and drug plate information, so that every plate on a single day of imaging has a unique plate id, for example L01_s01_01 where:

L01-04 - library plate number (out of 4 library plates)
sh01-03 - shuffle number (out of the 3 shuffled stock plates)
01-13 - imaging run number for that day

17 Remove the shuffled library plates (stock) plates from the **-20 °C** freezer, allow to thaw at room temperature and spin to collect contents at bottom of wells

18 Pre-label the appropriate (11) skirted 96 well plates with library plate IDs (L01-L04_sh01-sh03) to make up diluted drug plates

19 Dispense **19.6 µl** water into each well of the prelabeled dilution plates using multichannel pipette and reagent reservoir

20 Using VIAFLO (hedgehog) dispenser on BG_STOCK custom program, premix drug in drug library plates in slot A, and then transfer **1.4 µl** drug in DMSO to the dilution plates prefilled with water in slot B. Repeat for all drug library plates

Double check the dispense volumes before making up the diluted plates

Z-heights have been configured in this program to prevent pipette and plate crashes

21 Using VIAFILL (octopus) dispenser, dispense **5 µl** water onto 5 x predried imaging plates

5 plates at a time to prevent the agar absorbing all the liquid before the drug is dispensed into the water droplet

22 Using VIAFLO in custom program BC_AGAR, with correct drug library plate in slot B, transfer **3 µl** of diluted drug

Citation: Ida Barlow, Adam Mcdermott-Rouse, Luigi Feriani (10/28/2020). Syngenta divergent strain screen protocol. https://dx.doi.org/10.17504/protocols.io.bn5zmg76

and water mixture onto the correctly labelled imaging plate in slot A.

Repeat until all imaging plates have had drug dispensed onto them.

Z-heights have been configured in this program to prevent pipette tips from piercing the agar

23 Prepare 1:10 dilution of OP50 in M9 in a small bottle:

5 mL OP50

45 mL M9

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

25 Place lids on each plate and leave drugged and seeded imaging plates overnight at room temperatue in the dark (with box ontop)

Refeed L1s for day 2 of tracking (-1 days for day 1 of tracking)

26 At 17:00, spin L1s for day 2 of tracking at 2500rpm. Remove supernatent and using glass pipette, drop 4 small droplets around the edges of the plate (off food) onto 3 x 150mm plates per strain.

Allow to grow at **20 °C**

Day 1 imaging

27 Wash worms off 150mm paltes with M9 buffer using pasteur pipette into 15ml falcons

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

29 Remove supernatenet and fill with M9

30 Repeat steps 28-29

31 After final wash, fill falcon with M9 and transfer contents from 15ml falcon to 2 x 50ml and fill up 30ml with M9

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

Citation: Ida Barlow, Adam Mcdermott-Rouse, Luigi Feriani (10/28/2020). Syngenta divergent strain screen protocol. https://dx.doi.org/10.17504/protocols.io.bn5zmg76

32 YYYYMMDD_wormsorter.csv to plan and determine which strains to dispense into each plate.

Each strain will sequentially be dispensed into 10.5 imaging plates.

33 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

34 Expose worms to drug for 4 hours in total as calculated from the middle wormsorter time and allow worms to acclimate for 30 minutes in the cave prior to imaging

Example:
wormsorter start time 10 :00
wormsorter end time 11:00
middle wormsorter time 10:30

cave time 12:00
Imaging start time 12:30

35 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)

Prepare imaging plates for day 2 of imaging (on same day as day 1 of imaging)

36 Take 65-70 poured 96 well plates from the cold room, and weigh three random plates without their lids

37 Place in cabinet dryer (setting 1.5-2) and allow to dry for 2-3 hours with lids off

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

39 leave overnight at room temperature with lid

Perpare drug imaging plates for day 2 of imaging (+1 from day 1 of imaging)

40 Repeat steps 16-25 for the other 6 strains

Day 2 imaging

Citation: Ida Barlow, Adam Mcdermott-Rouse, Luigi Feriani (10/28/2020). Syngenta divergent strain screen protocol. https://dx.doi.org/10.17504/protocols.io.bn5zmg76

41 Repeat steps 27-35

Citation: Ida Barlow, Adam Mcdermott-Rouse, Luigi Feriani (10/28/2020). Syngenta divergent strain screen protocol. https://dx.doi.org/10.17504/protocols.io.bn5zmg76