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Prestwick screen protocol

Forked from Prestwick screen protocol

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1 *Works for me* This protocol is published without a DOI.

Tobrien

SUBMIT TO PLOS ONE

ABSTRACT

Protocol for screening the Prestwick *C. elegans* drug library of 240 drugs at 3 concentrations and imaging under baseline and bluelight conditions using the Hydra (Loopbio) imaging rigs. All drugs at all concentrations were imaged with N2 in a single day of tracking. This protocol was repeated 3 times so that there were 12 replicates per drug per concentration.

PROTOCOL CITATION

Ida Barlow, Thomas O'brien 2021. Prestwick screen protocol. **protocols.io** https://protocols.io/view/prestwick-screen-protocol-bs6znhf6

FORK NOTE

FORK FROM

Forked from Prestwick screen protocol, Ida Barlow

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

1 Pick 10 x N2 L4s onto 8 x 90mm plates (pre-seeded with OP50)

Pour 96WPs (up to 3 days before tracking) 4h

2 Prepare 1L low peptone NGM and autoclave

Making low peptone NGM for imaging plates
by Ida Barlow
PREVIEW RUN

2.1
- Book the autoclave (notebook on top of the machine).
- Take clean flasks from the glass kitchen.
- Measure all the pre-autoclave reagents and add to the flask (Use a new weighing boat and spatula for each reagent. Also, the cholesterol is kept in the fridge.)
- Once water is added mix thoroughly and label with autoclave tape ('Low peptone NGM Rm 5020'). Makesure the bottle is not screwed completely when placing it inside the autoclave machine.

2.2
- Turn ON the autoclave
- Make sure that the autoclave's probe bottle is the same size as the largest bottle you use and fill it with water.
- Place the temperature probe in it.
- Fill up the autoclave with water until it reaches the grill.
- Place the bottles in the autoclave and make sure that the cap is not screwed completely.
- Check the waste flask is not too full
- Use 'media' program.
- Press START.
- It will take about 2 hours for a 500ml bottle to autoclave and about 2.5 hours for 1L or larger bottles.

- When autoclave is complete, remove the probe flask

2.3
- Make sure to wear gloves as the flask will be hot
- Let the agar to cool to around 55°C, ie the bottle is cool enough to hold for a second with a gloved hand.
- Add the post autoclave reagents.
- Mix it well and start pouring onto imaging plates (See Protocol for plate pouring)
- Try not to shake the bottle too much while mixing to avoid air bubbles.
- The agar needs to be warm to be poured without blocking the tubings, so try to pour as quickly as possible and if not poured immediately put the bottle on a waterbath set to 60C until being used.

3 Once agar has cooled to around **55 °C** , add the salts and dispense agar into 40 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**

Dispensing agar into multiwell plates
by Saul Moore
PREVIEW RUN

3.1 Prepare a 250ml bottle of hot milliQ water in the microwave and keep in the waterbath along with the agar. The water is important to have on hand in case of tubing blockages.

3.2 Insert large cassette into the machine

3.3 Configure X, Y, and Z settings for the multiwell plate by clicking on tool symbol -> stage alignment.

For UNIPLATE96SQWLF 650U:

X = 95.6
Y = 4.2
Z = -22.5

For 48WP:

3.3.1 Put the plate into the stage and then press 'Move' so that the plate moves so that it is under the dispensing cassette.

3.3.2 Use the up and down arrows to move the pipette tip so that they hover just over the plate and make note of the height (this will be entered into the dispensing program at a later step). Press 'Fast/Slow' button to switch between fast and slow movements.

3.3.3 Use the X, Y arrows to move the plate so that the pipette tips are centered in the middle of column 5.

3.3.4 Save all settings.

3.4 Exit settings by pressing the back button

3.5 Press on the program you wish to use (see later for configuring your own program)

3.6 Make sure that the correct cassette is listed and change if necessary

3.7 Select the volume you wish to dispense

For 96WP:
200 µL

3.8 Select 'set height' and set the appropriate height for tip height (usually all the same)

3.9 Place the end of the tubing from the casettee into the agar that is being kept warm in the water bath

3.10 Press 'Prime' to prime the tubing and allow to finish so that agar flows from the pipette tips.

IMPORTANT:
Once the agar is in the tubing it is important to act quickly to avoid agar solidifying and causing blockages. If you are particularly concerned about agar cooling in the tubing, wrap the tubing in aluminium foil to keep hot.

Step 3.10 includes a Step case.
Unblocking the tubing

step case

Unblocking the tubing

If the tubing does block, clear the blockage by 'reverse priming' as much of the agar as possible.

Then place tube ends in the hot water and prime continuously with hot water until the water runs all the way through.

If you are having trouble getting the water through, squeeze and massage parts of the tubing where you can see blockages to force the agar along and allow the water to pass.

Once all cleared, 'reverse prime', and reprime with the agar

3.11 Place a clean plate in the stage

3.12 Press run and then plate should fill with agar

3.13 Repeat steps 11-12 until all the plates have been filled.

Little drops of agar can solidify on the tip ends. It is often good to remove these drops using a pipette tip every few runs so that blockages do not occur.

3.14 'Reverse prime' all the agar

3.15 Place the tubing ends into the hot water.

3.16 Prime so that the water runs through and clears all the agar

3.17 Reverse prime to remove the water

3.18 Release tension from the tubing and remove cassette

3.19 Double wrap the cassette in aluminium foil for autoclaving

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

4 Dry 15 x 150mm plates in cabinet dryer (setting 2) for 3 hours

5 Seed 15 x 150mm plates with OP50 and leave to dry overnight 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

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

6.18 Remove supernatant with plastic Pasteur pipette

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^{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 L1s (-3 days) 4h

7 At 11:00, 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 14 x 150mm plates

Allow to grow at **20 °C**

See above Step (6) for bleach synchronisation of C. elegans.

Dry plates and dispense drugs onto imaging plates usinv VIAFLO (-1 days from imaging) 3h

8 In the morning, take 40 poured 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 Pre-label dried 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 P01_sh01_01 where:

P01-09 - library plate number (out of 4 library plates)
sh01-04 - shuffle number (out of the 4 shuffled stock plates)
01-07 - imaging run number for that day

12 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

13 Pre-label the skirted 96 well plates with library plate IDs (P01-L09_sh01-sh04) to make up diluted drug plates

14 Dispense **7 µl** water into each well of the prelabeled dilution plates using multichannel pipette and reagent reservoir

15 Using VIAFLO (hedgehog) dispenser on BG_STOCK custom program, premix drug in drug library plates in slot A, and then transfer **0.5 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

16 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

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

18 Leave plates to dry for approx 15 mins, so all drugs are absorbed into the agar.

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

5 mL OP50

45 mL M9

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

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

Imaging

22 Wash worms off half (7) of the 150mm plates with M9 buffer using pasteur pipette into 15ml falcons

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

24 Remove supernatent and fill with M9

25 Repeat steps 23-24

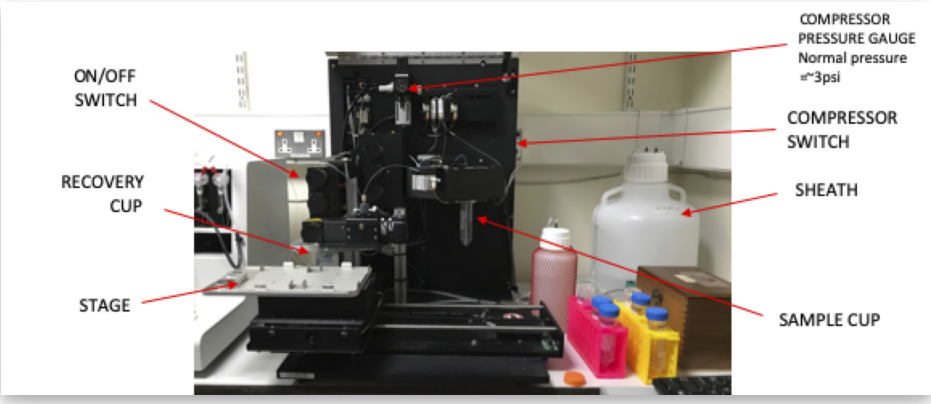
26 After final wash, fill falcon with M9 and transfer contents from 15ml falcon to 3 x 50ml and fill up 30ml with M9

27 Use COPAS wormsorter to dispense 3 worms per well (pure, no double) into 5 imaging plates at a time. Use pre-made YYYYMMDD_wormsorter.csv to plan and determine which plates to dispense onto

COPAS wormsorter
by Ida Barlow

PREVIEW RUN

27.1



COPAS wormsorter indicating key components

Turn on the compressor at the wall – it should show a pressure of 40psi after switched on

27.2 Turn on COPAS machine with switch on the left hand side

27.3 Turn on the lasers (488 laser sufficient if using unmarked animals). Add in picture of lasers.

27.4 Turn on the computer

27.5 Discard waste contents that are in the recovery cup (small shallow cup on the left-hand side of the machine)

27.6 Check that there is water in the sheath. If the water is low, fill up with MQH2O (not M9).

27.7 Make sure that the recovery cup and sample cup are securely tightened so that there are no leaks in the system

27.8 Open dbgview – should always be running in the background

27.9 Open FlowPilot software and a prepared experiment with a set gate for eg Adults. :

27.9.1 File -> Load Experiment

27.9.2 File -> Load sample

7.10 Maintenance -> Flush Sample

7.11 Click 'Refill Sample' – the sample cup pressure should decrease. You can see this in the software on the left hand size (include screenshot).

Sometimes the sample cup pressure doesn't decrease and in fact increases. You can still unscrew the sample cup but if this persists there may be a blockage.

7.12 Unscrew sample cup and replace with falcon filled with cleaning solution (pink in colour)

7.13 Once securely replaced click ‘Done refill’

7.14 Check ‘Sample on’ and ‘mixer on’ – cleaning solution should now pass through the system; allow a 2-3 ml to pass through (make sure sheath is unchecked)

> You will get a warning about contaminating the flow cell, this normal and you can click 'Yes'

7.15 Uncheck ‘Sample on’ or click Abort to stop sample flow.

7.16 Repeat steps 11-15 with water

7.17 Repeat steps 11-13 with sample.

7.18 **Turn mixer ON**. If you do not do this you may lose all your worms that have settled to the bottom of the tube!!!

7.19 Maintenance -> Prime Flow Cell; to flush sample through the system and remove air bubbles

7.20 Maintenance -> Flush sample

7.21 Check 488nm (and 568nm) laser boxes

7.22 Check ‘Use sort gate’ for stored sort gate – include screenshot of software here

7.23 Click ‘Acquire’ – sample should pass through the system and number of events per second will be shown:
- Aim for 10-20 events per second
- If too few/too many events increase/decrease ‘Sample cup pressure’ so that it is between 1.5-2psi
- To ensure only one event per droplet go to Setup->Coincidence, select ‘Pure, no double’. This increases accuracy in the number of worms dispensed but the time to dispense may increase.

7.24 Click on the plate icon on the top bar

7.25 Select number of objects to sort

7.26 Select the wells you would like to fill (for testing we use a spare 60mm plate and fill wells A1, A2, B1, B2

7.27 Select which gate to use

7.28 Apply

7.29 Place 60mm plate in front left corner of left-hand stage with A1 in the left corner.

7.30 Click ‘Fill plate’

7.31 Keep an eye on the number of events per second

7.32 Ensure the ‘Diverter pressure’ is checked

7.33 Check under microscope that the correct number of objects were dispensed per ‘well’

7.34 If too many objects, decrease sample cup pressure and repeat steps 8-11 or select Pure no double to increase accuracy.

7.35 Click on the plate icon on the top bar

7.36 ‘Clear plate’

7.37 Select number of objects per well and click ‘Apply to All’ or select which wells you would like to fill.

7.38 Apply

7.39 Place 96 well plate in left-hand stage

7.40 Ensure 'Diverter pressure' is checked'; if it is not then liquid comes out of the dispenser constantly and you get flooding.

7.41 'Fill plate'

7.42 Keep an eye on the number of events per second still and monitor how much sample fluid is coming through the system

7.43 Repeat steps 11-15

7.44 Keep sample cup with water secured so that the system is air-tight and closed

7.45 Turn off all equipment (Computer, lasers, compressor, worm sorter).

28 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 **20 °C** incubator

29 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 10:40
middle wormsorter time 10:20

cave time 13:50
Imaging start time 14:20

30 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)

```
python3 ~/scripts/run_syngenta_experiment_v2.py
```

31 After 15 plates have been filled with this batch of N2s, repeat steps 23-30 with the other batch of N2s on 150mm plates and record which batch was used in each plate in the wormsorter.csv file.