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Ida Barlow¹, Adam McDermott-Rouse¹, Luigi Feriani¹

¹Imperial College London

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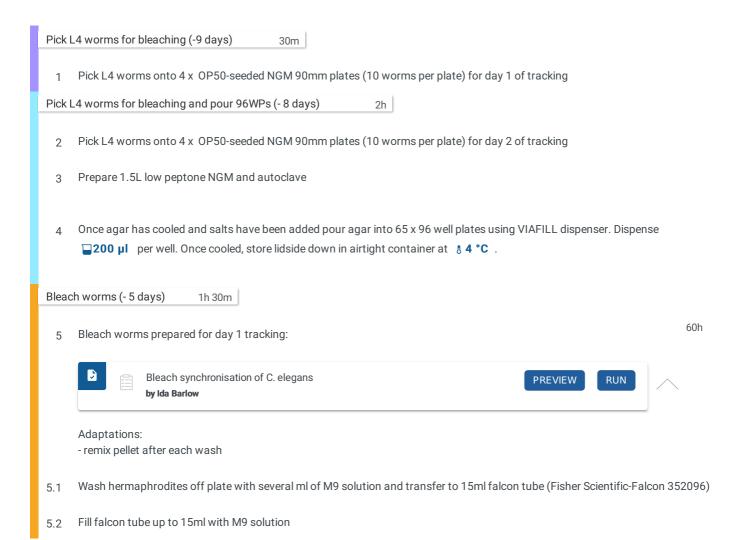


ABSTRACT

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

MATERIALS

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



- 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

- After final wash add 15ml M9 and store eggs/larvae in the falcon on the rotator that is constantly spinning at 20°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 8 20 °C in rotatotor until refeeding:



Adaptations:

- remix pellet after each wash
- 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

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Refeed L1s (-3 days)

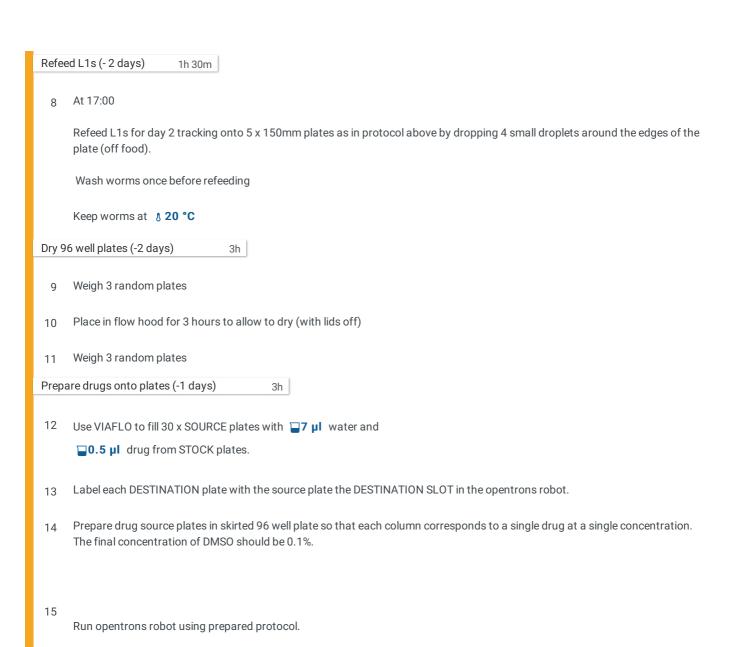
1h 30m

7 At 17:00

Refeed L1s for day 1 tracking onto $5 \times 150 \text{mm}$ 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



NID

- 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

```
Protocol parameters:
15.1
        # 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
       H20_source_slot = '9'
       H2O_source_type = 'trough-12row'
       H2O_source_well = 'A1'
       H20_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)
       Make sure all labware is loaded correctly
15.2
        Run protocol and monitor robot to ensure all the tips are removed
15.3
       Tip racks have to replaced after each destination plate is filled
15.4
     Allow the plates to dry for 30 minutes
16
Seed plates (-1 days)
17
      Use VIAFILL dispenser to dispense 5 µl 1:10 diluted OP50 into each well of each drug plate
      Allow to dry for 30 minutes under the flow hood
18
      Keep at room temperature over night (covered in the dark as some drugs are light-sensitive)
19
Disepensing worms
      Wash worms off the 150mm plates with M9 using a pasteur pipette into 15ml falcons
```

- 21 Spin at **31500 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

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