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COPAS wormsorter V.1

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Saul Moore[1], Ida Barlow[1]

[1]Imperial College London

Behavioural Genomics

Ida Barlow

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

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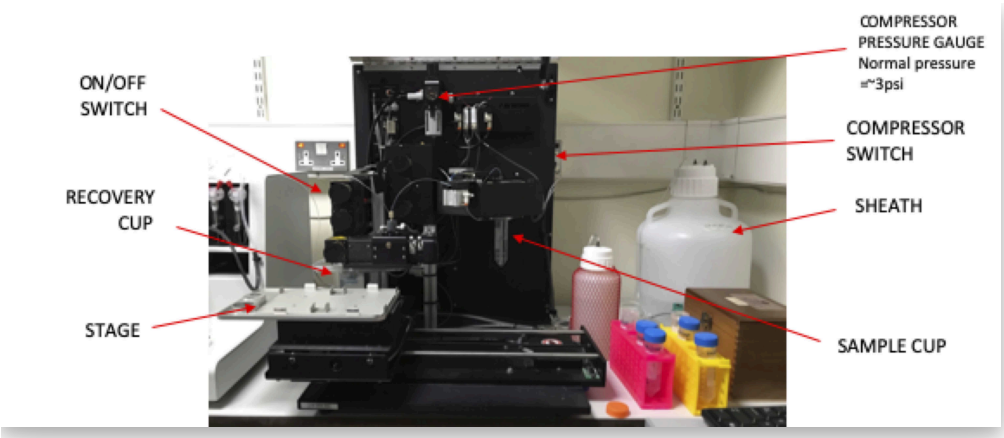
Abstract

Protocol for dispensing adult worms using the COPAS 500 flowpilot

Troubleshooting

Prepare equipment

1



COPAS wormsorter indicating key components

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

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

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

4 Turn on the computer

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

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

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

Prepare software

8 Open dbgview – should always be running in the background

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

9.1 File → Load Experiment

9.2 File → Load sample

Clean system

10 Maintenance → Flush Sample

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

> Note
>
> 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.

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

13 Once securely replaced click 'Done refill'

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)

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

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

16 Repeat steps 11-15 with water

Load sample

17 Repeat steps 11-13 with sample.

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!!!

19 Maintenance → Prime Flow Cell; to flush sample through the system and remove air bubbles

20 Maintenance → Flush sample

21 Check 488nm (and 568nm) laser boxes

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

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.

Test dispensing

24 Click on the plate icon on the top bar

25 Select number of objects to sort

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

27 Select which gate to use

28 Apply

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

30 Click ‘Fill plate’

31 Keep an eye on the number of events per second

32 Ensure the ‘Diverter pressure’ is checked

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

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

Fill plate

35 Click on the plate icon on the top bar

36 ‘Clear plate’

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

38 Apply

39 Place 96 well plate in left-hand stage

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

41 ‘Fill plate’

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

Clean system

43 Repeat steps 11-15

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

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