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COPAS wormsorter v.2

Forked from a private protocol

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Behavioural Genomics

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ABSTRACT

Protocol for dispensing adult worms using the COPAS 500 flowpilot

DOI

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PROTOCOL CITATION

Saul Moore, Ida Barlow 2021. COPAS wormsorter v.2. **protocols.io** https://dx.doi.org/10.17504/protocols.io.bvkbn4sn

FORK NOTE

FORK FROM

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50531

PARENT PROTOCOLS

In steps of

Deep cleaning COPAS

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Prepare equipment

1

Layout of sorter

Turn on equipment in the following order
a.Sorter (left side of unit near bottom)
b.Computer and monitor
c.Laser, located under computer
d.Vacuum pump (switch on wall to right of sorter)

2 Discard waste contents that are in the recovery cup (small shallow cup on the left-hand side of the machine)
a.Check/clean the recovery cup filter
b.Replace filter ~once per week with a new cell strainer (it should clip into place)

3 Check that there is water in the sheath. If the water is low (less than 5cm above the top of the green sensor), fill up with**sterile H_2O (from 4thfloor kitchen)**

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

5 Check that there is sterile water in the sample cup

6 Check for stray plate lids etc in the area around the COPAS stage and remove

Prepare software

7 Open dbgview – should always be running in the background

8 Open FlowPilot software:
a.File -> Load Experiment
i.Select appropriate template experiment from Documents/BehaviouralGenomics (adults, L4s, L2s all have different gates set)
b.File -> Load sample (will load the sample with the appropriate gate for your experiments)

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9 Save your experiment for this day of sorting (YYYYMMDD_experimentname)

10 Save sample (adults/L4s etc)

11 1. Check that the pressures are all okay (bottom left of software screen):

Compartment	Pressure (psi)
Sheath	~2.6
Sample cup	~1.6
Diverter	6
Cleaning	4

Clean system

12 Maintenance -> Flush Sample

13 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.

14 Unscrew sample cup and replace with 50ml falcon filled with 50ml 20% Decon-90 (D901-04; dilute in sterile water)

15 Once securely replaced click 'Done refill'

16 Check 'Sample on' and 'mixer on' – Decon solution should now pass through the system; allow a 50 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'

17 Uncheck 'Sample on' or click Abort to stop sample flow.

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18 Repeat steps 13-17 with cleaning solution (Product Number: 300-5072-000; pink in colour) but only pass 10ml through the system

19 Repeat steps 13-17 with a falcon filled with 50ml sterile water (from 4thfloor kitchen) and pass >25ml through the system

Flow check

20 Hold an empty 50ml faclon under the sample dispenser (otherwise you will end up with a puddle of water on the COPAS!)

21 Maintenance -> Flow check

22 Allow the water to flow into the falcon tube for the full 60 seconds

23 Record volume in the COPAS logsheet (Volumes/behavgenom$/Documentation/Protocols/COPAS_wormsorter/COPAS_logs/COPAS_logbook.xlsx)
a.Aim for flow of 25ml/minute

Control particles

24 Prepare 8mL solution of 1X control particles (Product number: 310-5071-001; 2 mL 4X stock solution, 6mL sterile water; label tube with date and wrap in foil to keep in the dark) – to be prepared once per week

25 Set up -> Control Particles

26 Acquire

27 Adjust Sample Cup pressure so that you have 10-20 events/sec

28 Aim for TOF Cv around 10-15% and tight narrow peak for TOF and Extinction

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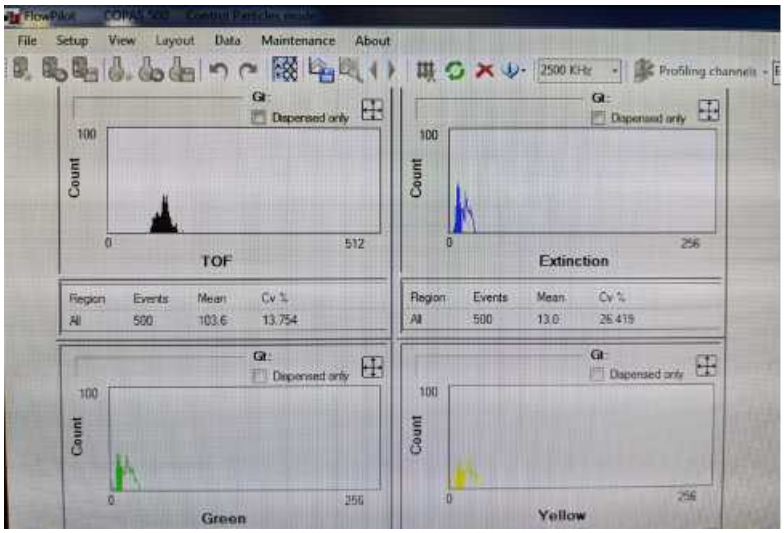

29 Record TOF and Extinction mean and cv in Volumes/behavgenom$/Documentation/Protocols/COPAS_wormsorter/COPAS_logs/COPAS_logbook.xlsx

30 Take a screenshot of the control particles screen and save into Documents/BehaviouralGenomics/ControlParticles
a.Print Screen
b.Open Paint
c.Ctr + v
d.Save

31 Set up -> control particles to exit

32 Refill sample with sterile water, run 10 mL water. You are now ready to load your sample

Load sample

33 Refill sample

34 Gently swirl falcon with worms and screw into sample cup

35 Done refill

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

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

38 Maintenance -> Flush sample

39 Check 488nm (and 568nm) laser boxes

40 Check 'Use sort gate' for stored sort gate

41 Click 'Acquire' – sample should pass through the system and number of events per second will be shown:
a.Aim for 5-20 events per second
b.If too few/too many events increase/decrease 'Sample cup pressure' so that it is between 1.5-2psi
c.Gate recovery should be between 20-50%
d.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

42 Click on the plate icon on the top bar

43 Select number of objects to sort

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

45 Select which gate to use (R4)

46 Apply

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

48 Click 'Fill plate' (the sheath will automatically turn on)

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49 Keep an eye on the number of events per second

50 Ensure the ‘Diverter pressure’ is checked

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

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

Fill plate

53 Click on the plate icon on the top bar

54 ‘Clear plate’

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

56 Apply

57 Place 96 well plate in left-hand stage (A1 aligned)

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

59 ‘Fill plate’

60 Each time you do a dispense, save the file in the convention: YYYYMMDD_strain_ _platenumber in Documents/yourname/experimentname/date (eg. 20210211_PHX1675_S01R01)

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

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Clean system

62 Repeat steps 13-19

63 Keep sample cup with 50ml water in the falcon secured so that the system is air-tight and closed

64 Empty recovery cup and waste bottle on floor

65 Remove waste tray (see below), unscrew, and empty and spray with water

66 Turn off all equipment in this order:
a.Software
b.Sorter
c.Pump
d.Computers
e.Lasers

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