

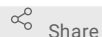


Jun 07, 2021

# Deep cleaning COPAS

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1 Works for me



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Behavioural Genomics

Bonnie Evans

## ABSTRACT

To prevent gradual build-up of slime-mould and other contaminants in the COPAS tubing (particularly in the tubing from the sheath), that cause blockages in the flow cell and also lead to erroneous Extinction/TOF readings, the COPAS machine should be 'Deep Cleaned' at least once per month (every 2 weeks if in regular use)

## DOI

[dx.doi.org/10.17504/protocols.io.bvkan4se](https://dx.doi.org/10.17504/protocols.io.bvkan4se)

## PROTOCOL CITATION

Saul Moore 2021. Deep cleaning COPAS. **protocols.io**  
<https://dx.doi.org/10.17504/protocols.io.bvkan4se>



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## CREATED

Jun 07, 2021

## LAST MODIFIED

Jun 07, 2021

## PROTOCOL INTEGER ID

50530

## MATERIALS TEXT

10 x 2L Sterile Water  
1L Decon-90

## BEFORE STARTING

Deep cleaning will take 3 hours – hands-on time is around 1 hour with checks every 20 minutes in between

### Pre-sorting clean

- 1 Follow COPAS Protocol up to end of 'Pre-sorting clean' (steps 1-19):



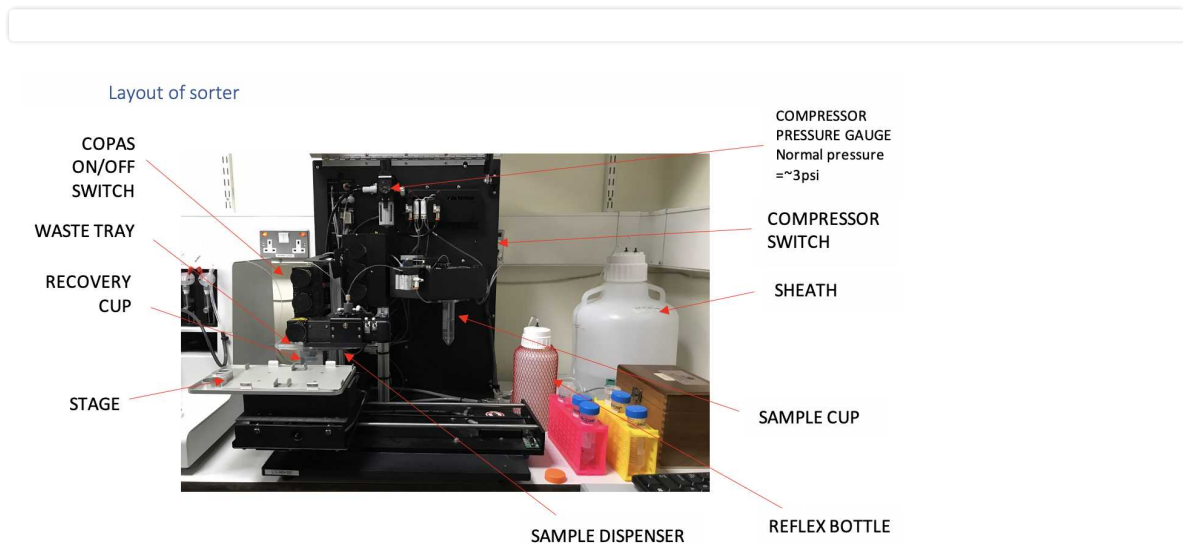
COPAS wormsorter v.2  
by Bonnie Evans

PREVIEW

RUN



## 1.1



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)

- 1.2 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)
- 1.3 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<sub>2</sub>O (from 4<sup>th</sup> floor kitchen)**
- 1.4 Make sure that the recovery cup and sample cup are securely tightened so that there are no leaks in the system
- 1.5 Check that there is sterile water in the sample cup
- 1.6 Check for stray plate lids etc in the area around the COPAS stage and remove
- 1.7 Open dbgview – should always be running in the background
- 1.8 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)

1.9 Save your experiment for this day of sorting (YYYYMMDD\_experimentname)

1.10 Save sample (adults/L4s etc)

1.11 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

1.12 Maintenance -> Flush Sample

1.13 Click 'Refill Sample' – the sample cup pressure should decrease. You can see this in the software on the left hand side (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.

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

1.15 Once securely replaced click 'Done refill'

1.16 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'

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

1.17

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

1.19 Repeat steps 13-17 with a falcon filled with 50ml sterile water (from 4<sup>th</sup> floor kitchen) and pass >25ml through the system

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

1.21 Maintenance -> Flow check

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

1.23 Record volume in the COPAS logsheet  
(Volumes/behavgenom\$/Documentation/Protocols/COPAS\_wormsorter/COPAS\_logs/COPAS\_logbook.xlsx)  
a. Aim for flow of 25ml/minute

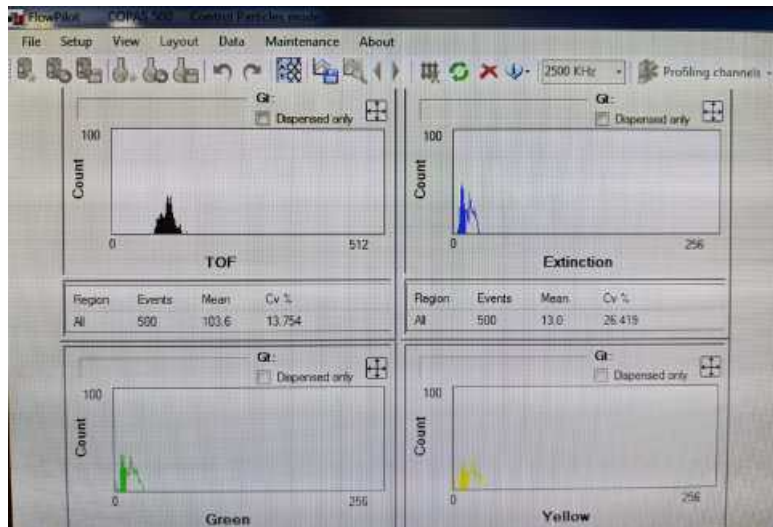
1.24 Prepare 8mL solution of 1X control particles (Product number: 310-5071-001; 2 mL 4X stock solution, 6mL sterile water; label tube with date and wrap in foil to keep in the dark) – to be prepared once per week

1.25 Set up -> Control Particles

1.26 Acquire

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

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



- 1.29 Record TOF and Extinction mean and cv in  
Volumes/behavgenom\$/Documentation/Protocols/COPAS\_wormsorter/COPAS\_logs/COPAS\_logbook.xlsx
- 1.30 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
- 1.31 Set up -> control particles to exit
- 1.32 Refill sample with sterile water, run 10 mL water. You are now ready to load your sample
- 1.33 Refill sample
- 1.34 Gently swirl falcon with worms and screw into sample cup
- 1.35 Done refill
- 1.36 **Turn mixer ON.** If you do not do this you may lose all your worms that have settled to the bottom of the tube!!!

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

1.37

1.38 Maintenance -> Flush sample

1.39 Check 488nm (and 568nm) laser boxes

1.40 Check 'Use sort gate' for stored sort gate

1.41 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.

1.42 Click on the plate icon on the top bar

1.43 Select number of objects to sort

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

1.45 Select which gate to use (R4)

1.46 Apply

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

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

1.49 Keep an eye on the number of events per second

- 1.50 Ensure the 'Diverter pressure' is checked
- 1.51 Check under microscope that the correct number of objects were dispensed per 'well'
- 1.52 If too many objects, decrease sample cup pressure and repeat steps 8-11 or select Pure no double to increase accuracy.
- 1.53 Click on the plate icon on the top bar
- 1.54 'Clear plate'
- 1.55 Select number of objects per well and click 'Apply to All' or select which wells you would like to fill.
- 1.56 Apply
- 1.57 Place 96 well plate in left-hand stage (A1 aligned)
- 1.58 Ensure 'Diverter pressure' is checked'; if it is not then liquid comes out of the dispenser constantly and you get flooding.
- 1.59 'Fill plate'
- 1.60 Each time you do a dispense, save the file in the convention: YYYYMMDD\_strain\_\_platenumber in Documents/yourname/experimentname/date (eg. 20210211\_PHX1675\_S01R01)
- 1.61 Keep an eye on the number of events per second still and monitor how much sample fluid is coming through the system
- 1.62 Repeat steps 13-19

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

1.64 Empty recovery cup and waste bottle on floor

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



1.66 Turn off all equipment in this order:

- a. Software
- b. Sorter
- c. Pump
- d. Computers
- e. Lasers

#### DECON

- 2 Click 'Refill Sample' – this is to decrease the pressure in the system
- 3 Unscrew the sheath bottle lid, and disconnect the water sensor and the sheath tubing from the sheath bottle (press down on the silver catch)
- 4 Empty the contents of the sheath into the communal sink
- 5 Rinse sheath bottle with Sterile water
- 6 Fill up the sheath with at least 5L of 20% solution of DECON-90 detergent.  
For 5L:  
- 4L Sterile water (from 4th floor kitchen)



- 1L DECON-90 (D901-04, 5L, product code: 10335650)

- 7 Ensure that the solution fills the sheath sufficiently above the sensor water level
- 8 Reattach the sheath tubing (consider using fresh tubing) and sensor, and screw back on the sheath bottle lid
- 9 Click 'Done Refill'
- 10 Ensure 'Sheath ON'
- 11 Click 'Sample ON', and solution from the sheath should now pass through the system (instead of from the sample cup)
- 12 Leave the sheath solution to pass through the system for at least an hour, checking every 20 minutes or so to ensure that the level of solution in the sheath does not fall below the sensor level. The rate depends on the sheath PSI. A higher sheath PSI (4-5) can help with cleaning the tubing, but is not recommended for extended periods of time (citation needed). The best method I found was to massage the tubing while sheath solution is flowing through to help dislodge contaminants.

#### Sterile water

- 13 Click 'Refill Sample' and disconnect the sheath tubing, sensor and lid like before
- 14 Dispose of the remaining solution in the sheath down the sink (while running water from the tap to dilute to solution further)
- 15 Rinse out the sheath thoroughly by swilling several (5-6) times with sterile water and disposing in the sink, until no detergent bubbles remain, then rinse several times more
- 16 Refill the sheath back up to at least 5L (half way) with sterile water
- 17 Reconnect the sheath tubing, sensor and lid
- 18 Repeat Steps 7 – 10 with water for an hour

- 19 After running water through the COPAS, ensure 'Sheath OFF'
- 20 Repeat the Pre-sorting clean from the WormSorterProtocol.docx

#### Flow check/control particles

- 21 Test run with flow check and Control Particles (see steps 22-29):



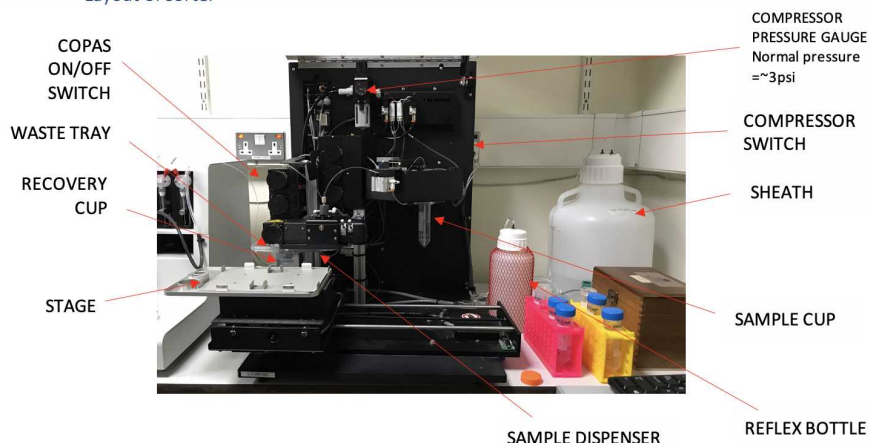
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PREVIEW

RUN

#### 21.1

Layout of sorter



Turn on equipment in the following order

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- c. Laser, located under computer
- d. Vacuum pump (switch on wall to right of sorter)

- 21.2 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)
- 21.3 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<sub>2</sub>O (from 4<sup>th</sup> floor kitchen)**
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- 21.8 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)
- 21.9 Save your experiment for this day of sorting (YYYYMMDD\_experimentname)
- 21.10 Save sample (adults/L4s etc)
- 21.11 1. Check that the pressures are all okay (bottom left of software screen):

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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.

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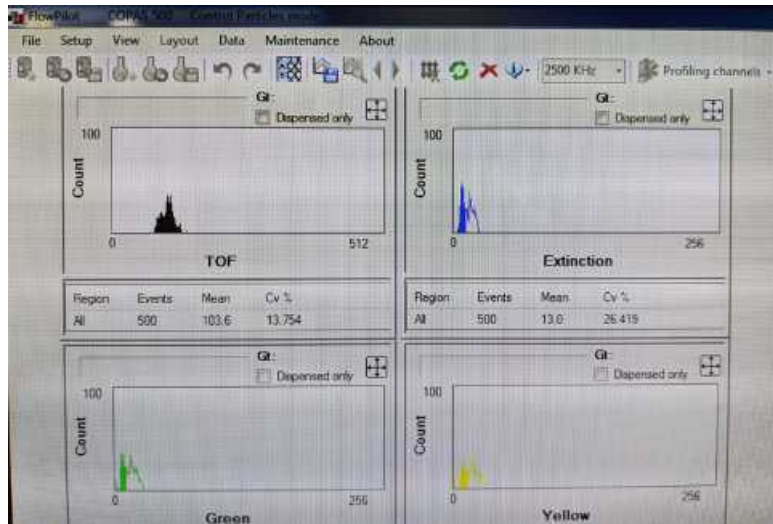
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