Keio Screen

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Feb 16, 2021

In Development This protocol is published without a DOI.

Behavioural Genomics


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SUBMIT TO PLOS ONE

ABSTRACT

Protocol for screening *Caenorhabditis elegans* behavioural response to bacterial gene-deletion mutants from the 'Keio Collection', to identify behaviour-modifying mutations for follow-up analyses.

PROTOCOL CITATION

Saul Moore 2021. Keio Screen. **protocols.io**
https://protocols.io/view/keio-screen-bnw7mfhn

KEYWORDS

Keio, deletion, mutant, knockout, C. elegans, behaviour, imaging, multiwell

CREATED

Oct 24, 2020

LAST MODIFIED

Feb 16, 2021

PROTOCOL INTEGER ID

43711

MATERIALS TEXT

Culture plates: Nunc™ 96-Well Polystyrene Round Bottom Microwell Plates, Non-Treated, 268200

Imaging plates: Whatman Square Well Flat Bottom UNIPLATE, 7701-1651

Preparing maintenance plates 1d

1 Prior to the experiment, prepare 35 NGM agar-filled 60mm Petri plates (worm maintenance plates).
To make 500mL NGM agar, follow the steps in the protocol for *Making normal NGM for imaging plates (Cabreiro Lab)* and pour 15ml NGM agar into each plate:
(Friday, -11 days)

Making normal NGM for imaging plates (Cabreiro Lab)
by Saul Moore
PREVIEW RUN

1.1
- 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 ('NGM Rm 5020'). Make sure the bottle is not screwed completely when placing it inside the autoclave machine.

1.2
- 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.

1.3
- When autoclave is complete, remove the probe flask
- 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 following the 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.

2

Inoculate 10ml LB broth media with BW25113 bacteria (used as negative control and for raising worms) for overnight culture, following the protocol, *Inoculating a Liquid Bacterial Culture,* and place in a shaking incubator at 37°C at 200 rpm:
(Friday, -11 days)

Inoculating a Liquid Bacterial Culture
by Priota Islam,
Imperial College London
PREVIEW RUN

2.1 Obtain LB Broth from the Media kitchen
LB Broth contents:
- 4 g NaCl
- 4 g Tryptone
- 2 g Yeast Extract
- dH_2O to 400 mL

2.2 Add liquid LB to a tube or flask and add the appropriate antibiotic (if required) to the correct concentration (see table below).

Antibiotic Concentrations

Commonly Used Antibiotics	Recommended Concentration
Ampicillin	100 µg/mL
Bleocin	5 µg/mL
Carbenicillin	100 µg/mL
Chloramphenicol	25 µg/mL
Coumermycin	25 µg/mL
Gentamycin	10 µg/mL
Kanamycin	50 µg/mL
Spectinomycin	50 µg/mL
Tetracycline	10 µg/mL

Antibiotic concentrations

Note: If you intend to do a mini-prep you will usually want to start 2 mL in a falcon tube, but for larger preps you might want to use as much as a litre of LB in a 2 L Erlenmeyer flask.

2.3 Using a sterile inoculation loop, select a single colony from your bacteria streaked LB agar plate.

2.4 Dip the inoculation loop into the liquid LB and swirl.
Discard the inoculation loop.

2.5 Loosely cover the culture with sterile aluminium foil or a cap that is not air tight as bacteria needs air.

2.6 Incubate the bacterial culture at the required growth temperature overnight (i.e. 12-18 hrs in general) in a shaking incubator.

2.7 After incubation, check for growth, which is characterized by a cloudy haze in the media.
Measure the optical density of the bacterial culture at 600nm wavelength using a spectrophotometer.
Record the OD600 three times and calculate average, use LB Broth as Blank.

3 Dry 10 maintenance plates for approximately 2 hours (depending on humidity).
(Saturday, -10 days)

4 

Seed the 10 maintenance plates with 120µL of BW25113 culture
(Saturday, -10 days)

5 Leave to dry on the bench top for about half an hour, and then store upside-down in an incubator at 25°C.
(Saturday, -10 days)

6 Using a platinum pick, gently pick 7 adult N2 Bristol *C. elegans* onto each of the 10 seeded maintenance plates, and store in an incubator at 20°C.
(Monday, -8 days)

7 Remove the adult worms after 24 hours, leaving the eggs behind to hatch into L1 larvae that will be raised on

BW25113.
(Tuesday, -7 days)

8

Inoculate 10ml LB broth media with BW25113 bacteria for overnight culture and place in a shaking incubator at 37°C, 200 rpm.
(Tuesday, -7 days)

9

Seed the remaining 25 maintenance plates with fresh BW25113 culture, and leave under hood until dry.
(Wednesday, -6 days)

10

Wash the worms off the 10 BW-seeded maintenance plates prepared in Step 4, into two 15ml Falcon tubes.
(Friday, -4 days)

11

Perform an egg prep on worms in the Falcon tubes, by following the protocol for *Egg Prep for Bleach Synchronization (Cabreiro Lab):*
(Friday, -4 days)

Egg Prep for Bleach Synchronization (Cabreiro Lab)
by Saul Moore
PREVIEW RUN

11.1 Wash worms off the plates with a few mL of M9 buffer into a 15mL Falcon tube.

11.2 Leave the Falcon tube to stand for a while until the worm settle to the bottom in a loose pellet.

11.3 Remove the supernatant leaving 2mL M9 solution in the tube (with the worms pelleted)

11.4 Mix together in an Eppendorf tube:

- **400 µl NaOH [4M]**
- **350 µl Sodium Hypochlorite [Acros Organics, 10/15% active chlorine, 7681-52-9]**

11.5 Add 350µL bleach mix to the 2mL solution of worms in M9

11.6 Vortex for 5 min (checking every 30 seconds under a microscope to see if the worms are broken apart and eggs have been released).

11.7 Once the majority of eggs have been released, quench the solution by topping up the Falcon tube to 14mL with M9.

11.8 

Centrifuge at 6700 rpm for 2 minutes to pellet the eggs to the bottom.

11.9 Carefully remove the supernatant using a plastic Pasteur pipette.

1.10 Top up the Falcon tube to 14mL with M9.

11.11 

Repeat Steps 8 - 10 three more times, to thoroughly quench the bleach solution.

1.12 After the final wash, top up to 10mL with M9.

1.13 Pipette the 10mL solution onto an empty 60mm plate using a glass Pasteur pipette, and incubate at 20°C.

1.14 The next morning, transfer the newly hatched L1 larvae in 10mL M9 solution to a sterile conical Eppendorf tube using a glass Pasteur pipette.

1.15 Centrifuge for 2 minutes at maximum speed (14,000 rpm).

1.16 Using a glass Pasteur pipette, remove as much supernatant as possible, and then pool all L1 worms together into a single Eppendorf tube.

1.17 Dispense 3µL of L1 solution on the lid of a 60mm Petri plate, and count under microscope how many worms are in the droplet. Use this to estimate the total worm concentration of the Eppendorf.

1.18 Using a DISTRIMAN Gilson repetitive pipette, dispense approximately 500 worms onto each plate.
NB: The concentration may be adjusted by re-centrifuging and removing less supernatant with the Pasteur pipette (to dilute the solution if needed), and re-counting the number of worms in 3µL.

12 The next day at about midday, wash the L1 larvae off the empty plate and re-feed onto the 25 newly seeded maintenance plates using a glass Pasteur pipette. Aim to dispense around 500 worms per plate.
(Saturday, -3 days)

Label 12 plates for harvesting L4 worms, and 13 plates for harvesting Day 1 adult worms, and incubate at 20°C.

13 (Saturday, -3 days)

Preparing NGM imaging plates (96WP)

14 Prepare 500ml NGM agar by following the steps in the protocol *Making normal NGM for imaging plates (Cabreiro Lab):*
(Wednesday, -6 days)

Making normal NGM for imaging plates (Cabreiro Lab)
by Saul Moore
PREVIEW RUN

14.1
- 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 ('NGM Rm 5020'). Make sure the bottle is not screwed completely when placing it inside the autoclave machine.

14.2
- 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.

14.3
- When autoclave is complete, remove the probe flask
- 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 following the 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.

15 Using the Integra ViaFill, dispense 200µL of NGM agar into each well of 8 imaging plates (Whatman Square Well Flat Bottom UNIPLATE), following the protocol *Dispensing agar into multiwell plates*:
(Wednesday, -6 days)

Dispensing agar into multiwell plates
by Saul Moore
PREVIEW RUN

15.1 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.

15.2 Insert large cassette into the machine

15.3 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:

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

15.3.2 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.

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

15.3.4 Save all settings.

15.4 Exit settings by pressing the back button

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

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

15.7 Select the volume you wish to dispense

For 96WP:
200 µL

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

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

5.10 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 15.10 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

5.11 Place a clean plate in the stage

5.12 Press run and then plate should fill with agar

5.13 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.

5.14 'Reverse prime' all the agar

5.15 Place the tubing ends into the hot water.

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

5.17 Reverse prime to remove the water

Release tension from the tubing and remove cassette

5.18

5.19 Double wrap the cassette in aluminium foil for autoclaving

16 Leave the plates on the lab bench (with lids on) until the agar is dry.
(Wednesday, -6 days)

17 Measure the weight of 3 plates (with lids on) and record average plate weight on day of pouring.
(Wednesday, -6 days)

18 Dry the plates under a hood (or drying cabinet) until the plates lose between 3-5% of their original plate weight (with lids on)
(Wednesday, -6 days)

19 Store the plates upside-down at 4°C (plates can be stored for up to 1 week prior to use).
(Wednesday, -6 days)

Inoculating from Keio stock plates (96WP)

20 

Fill the wells of two 96-well culture plates (Nunc™ 96-Well Polystyrene Round Bottom Microwell Plates, Non-Treated) with 200µL LB broth media and 50µg/ml Kanamycin.
(Wednesday, -6 days)

21 Remove Keio frozen library stock plate from -80°C, gently remove the aluminium film, and then leave the wells to defrost for a few minutes.
(Wednesday, -6 days)

22 

Inoculate the 96-well culture plates from the Keio stock plate, following the protocol for *Growing overnight bacterial culture in 96WP (Cabreiro Lab)*:
(Wednesday, -6 days)

Growing overnight bacterial culture in 96WP (Cabreiro Lab)
by Saul Moore
PREVIEW RUN

22.1 Obtain LB Broth from the Media kitchen

LB Broth contents:
4gNaCl
4 g Tryptone
2 g Yeast Extract
Add dH2O to 400 mL

22.2 Wipe the work area with 70% ethanol and create a sterile environment on the laboratory bench by using a bunsen or gas burner. Work under the hood if you have a large number of plates. Book the hood in advance in that case.

22.3 Label the inoculation plates and the lids with the corresponding frozen library plate ID

22.4 Add 200µl liquid LB to each well of the 96-well plate using a multichannel pipette and a sterile reservoir.
Details of the plates used:
Name: ThermoFisher Scientific Nunc™ 96-Well Polystyrene Round Bottom Microwell Plates, Non-Treated
Catalogue number: 268200

22.5 Take the frozen library plates out of the freezer just before doing the inoculation to avoid the wells thawing completely (We want to avoid repetitive freeze thawing of the bacterial strains). You can also use dry ice to take all the plates out of the freezer at once. In that case, just before inoculation take the respective plate off the dry ice and leave on bench top for 2mins

22.6 Position the plates so that well A1 of every plate is at the top left. You can also put sticker on the replicator to mark A1 and H12, to avoid confusion and contamination

22.7 To use the replicator, strap some laboratory/masking tape around your index and middle finger together with the adhesive part on the outer side. Use the stickiness of the tape to pick up the replicator by gently pressing on the surface

22.8 Carefully lower the sterile replicator into the frozen library plate, making sure to touch the surface or puncture into the frozen wells to obtain sufficient bacterial cells

22.9 Move the replicator immediately over to the inoculation plate containing LB and Kanamycin and dip the replicator into the liquid (try to swirl the replicator slightly while inside the wells to ensure the bacteria mixes with the liquid)

2.10 Wash the replicator with ethanol and then with water, and place inside an autoclavable box to be sterilised for later use (use a new replicator for every inoculation plate to avoid contamination)

2.11 Cover the inoculation plates with plastic lids and wrap in a damp tissue.

2.12 Place the bacterial cultures in an airtight box (to prevent evaporation from the wells) and incubate overnight at 37°C (no shaking).

2.13 After incubation, check for growth, which is characterized by a cloudy haze in the media

23 Wet some tissue with MilliQ water, and wrap the culture plates in the tissue.
NB: The tissue provides humidity that aids growth, while the presence of Kanamycin should prevent contamination.
(Wednesday, -6 days)

24 Incubate thee cultures overnight at 37°C (no shaking).
(Wednesday, -6 days)

25 Repeat Step 18 to prepare 2 more overnight culture plates.
(Thursday, -5 days)

26 Remove the overnight cultures from the incubator, and inoculate the newly prepared culture plates from yesterday's overnight culture plate using a replicator, following the above protocol for *Growing overnight bacterial culture in 96WP (Cabreiro Lab)*.
(Thursday, -5 days)

27 Repeat Step 21 to incubate overnight at 37°C (no shaking). These cultures ('twice removed' from frozen stocks) will be used for seeding the 'Old Lawn' imaging plates (ie. seeded 3 days prior to imaging and refrigerated).
(Thursday, -5 days)

28 Repeat Steps 18 - 21 to inoculate from Keio frozen stocks again for the 'New Lawn' imaging plates (ie. freshly seeded the day before imaging).
(Friday, -4 days)

29 Repeat Steps 22 - 24 for the second round of overnight cultures for the 'New Lawn' plates.
(Saturday, -3 days)

Seeding bacterial lawns (96WP)

30 Remove imaging plates from 4°C storage and record the average weight of three of the plates.
(Friday, -4 days)

31 Ensure that the imaging plates have lost approximately 3-5% of their original weight. Place under a hood (or drying cabinet) until this is the case.
(Friday, -4 days)

32 Remove overnight cultures for 'Old Lawn' plates that were prepared in Step 24, and seed 10µl bacterial culture into each well of 4 imaging plates using the Integra ViaFlo.
(Friday, -4 days)

33 Place seeded plates under a hood to dry for 20 minutes, then place in an incubator at 25°C (no shaking) for 7 hours 40 minutes (total lawn growth time: 8 hours).
(Friday, -4 days)

34 After 8 hours, remove the plates from the incubator and store overnight at 4°C.
(Friday, -4 days)

35 Remove overnight cultures for 'New Lawn' plates that were prepared in Step 26, and seed 10µl bacterial culture into each well of 4 imaging plates using the Integra ViaFlo.
(Sunday, -2 days)

36 Repeat Steps 30 - 31 for the 'New Lawn' plates.
(Sunday, -2 days)

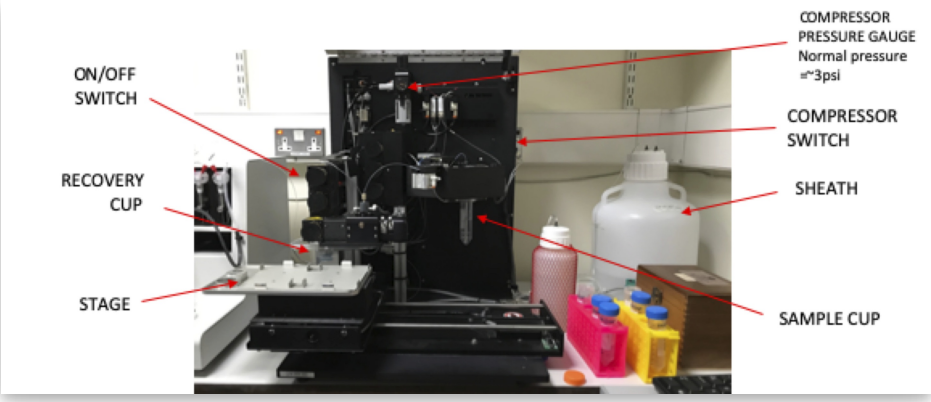
Dispensing worms using COPAS (96WP)

37 Remove 2 'New Lawn' and 2 'Old Lawn' seeded plates from 4°C, and dry for 30 minutes under a hood. Once dry, leave for a further 30 minutes to acclimate to room temperature.
(Monday, -1 day)

38 Turn on the COPAS machine by following Steps 1 - 16 of protocol *COPAS wormsorter* to prepare and clean the machine for dispensing L4-stage worms:
(Monday, -1 day)

COPAS wormsorter
by Ida Barlow
PREVIEW RUN

38.1



COPAS wormsorter indicating key components

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

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

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

38.4 Turn on the computer

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

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

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

38.8 Open dbgview – should always be running in the background

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

38.9.1 File -> Load Experiment

38.9.2 File -> Load sample

8.10 Maintenance -> Flush Sample

8.11 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.

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

8.13 Once securely replaced click 'Done refill'

8.14 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'

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

8.16 Repeat steps 11-15 with water

8.17 Repeat steps 11-13 with sample.

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

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

8.20 Maintenance -> Flush sample

8.21 Check 488nm (and 568nm) laser boxes

8.22 Check 'Use sort gate' for stored sort gate – include screenshot of software here

8.23 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.

8.24 Click on the plate icon on the top bar

8.25 Select number of objects to sort

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

8.27 Select which gate to use

8.28 Apply

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

8.30 Click ‘Fill plate’

8.31 Keep an eye on the number of events per second

8.32 Ensure the ‘Diverter pressure’ is checked

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

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

8.35 Click on the plate icon on the top bar

8.36 ‘Clear plate’

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

8.38 Apply

8.39 Place 96 well plate in left-hand stage

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

8.41 ‘Fill plate’

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

8.43 Repeat steps 11-15

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

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

39

Remove 12 maintenance plates with L4-stage worms (prepared in Step 11) and wash the worms off the plates into two 15ml Falcon tubes using approximately 10ml PBS 'A' buffer (sterile).
(Monday, -1 day)

40 Fill up the tubes to 15ml with PBA 'A' and centrifuge at 1000rpm for 2 minutes.
(Monday, -1 day)

41 Remove the supernatant using a Pasteur pipette.
(Monday, -1 day)

42

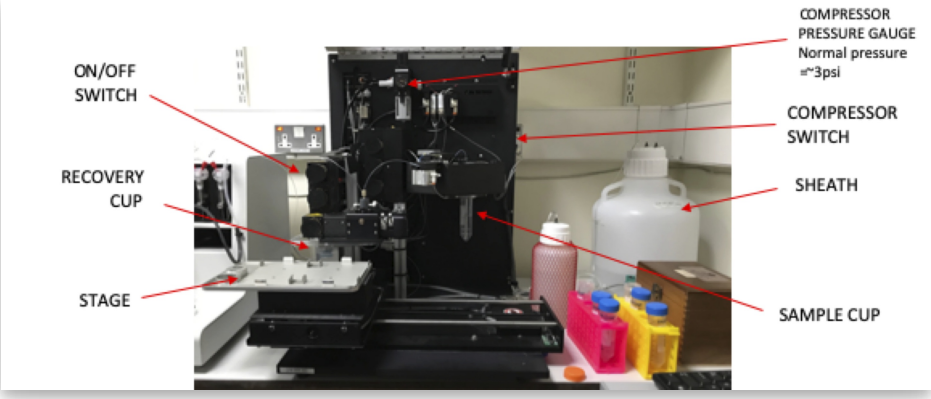
Repeat Steps 36 - 37 four more times to thoroughly rinse off any BW25113 bacteria.
(Monday, -1 day)

43 Re-suspend the worms and divide them equally into two 50ml Falcon tubes (for the COPAS), and fill them both up to approximately 40ml with PBS 'A'.
(Monday, -1 day)

44 Dispense 3 L4-stage worms into each well of the 4 imaging plates (2 'New', 2 'Old') using the COPAS following the protocol *COPAS wormsorter:*
(Monday, -1 day)

COPAS wormsorter
by Ida Barlow
PREVIEW RUN

44.1



COPAS wormsorter indicating key components

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

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

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

44.4 Turn on the computer

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

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

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

44.8 Open dbgview – should always be running in the background

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

44.9.1 File -> Load Experiment

44.9.2 File -> Load sample

4.10 Maintenance -> Flush Sample

4.11 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.

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

4.13 Once securely replaced click ‘Done refill’

4.14 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'

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

4.16 Repeat steps 11-15 with water

4.17 Repeat steps 11-13 with sample.

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

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

4.20 Maintenance -> Flush sample

4.21 Check 488nm (and 568nm) laser boxes

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

4.23 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.

4.24 Click on the plate icon on the top bar

4.25 Select number of objects to sort

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

4.27 Select which gate to use

4.28 Apply

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

4.30 Click ‘Fill plate’

4.31 Keep an eye on the number of events per second

4.32 Ensure the ‘Diverter pressure’ is checked

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

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

4.35 Click on the plate icon on the top bar

4.36 ‘Clear plate’

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

4.38 Apply

4.39 Place 96 well plate in left-hand stage

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

4.41 ‘Fill plate’

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

4.43 Repeat steps 11-15

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

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

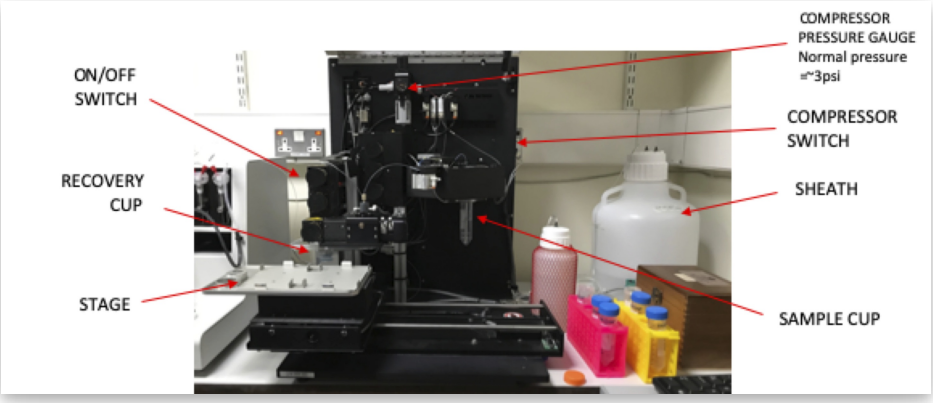
45 Leave the plates to dry under a hood for 30 minutes, then incubate at 20°C overnight.
(Monday, -1 day)

46 Repeat Steps 34 - 40 for the 13 remaining maintenance plates with Day 1 adult worms to prepare them for COPAS.
(Tuesday, Tracking Day 1)

47 Dispense 3 Day 1 adult worms into each well of the 4 imaging plates (2 'New', 2 'Old') using the COPAS following the protocol *COPAS wormsorter:*
(Tuesday, Tracking Day 1)

COPAS wormsorter
by Ida Barlow
PREVIEW RUN

47.1



COPAS wormsorter indicating key components

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

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

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

47.4 Turn on the computer

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

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

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

47.8 Open dbgview – should always be running in the background

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

47.9.1 File -> Load Experiment

47.9.2 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

Check 488nm (and 568nm) laser boxes

7.21

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).

48 Leave the plates to dry under a hood for 30 minutes before placing them in the imaging cave to acclimate for a further 30 minutes prior to tracking using the Hydra imaging rig.
(Tuesday, Tracking Day 1)

Tracking using Hydra rigs

49 Remove the imaging plates (with L4-dispensed worms) from the 20°C incubator and leave to acclimate in the imaging cave for 30 minutes prior to tracking (+24 hours and +25 hours on food, ie. after dispensing with COPAS in Step 41).
(On Tuesday, Tracking Day 1)

50 

Record L4-dispensed worm behaviour on the bacterial food for 15 minutes at the +24 hour and +25 hour timepoints (25 fps, exposure: 25000 msec, with blue-light stimulation).
(On Tuesday, Tracking Day 1)

51 

Record Day 1-dispensed worm behaviour on thee bacterial food for 15 minutes at the +1, +3, and +5 hour timepoints.
(On Tuesday, Tracking Day 1)

52 After imaging the +5 hour timepoint for Day 1-dispensed worms, store the Day 1-dispensed imaging plates in the 20°C incubator overnight.
(On Tuesday, Tracking Day 1)

53 Remove Day 1-dispensed imaging plates from the 20°C incubator 30 minutes prior to tracking +24 hour timepoint, and leave to acclimate in the imaging cave.
(On Wednesday, Tracking Day 2)

54 

Record Day 1-dispensed worm behaviour on thee bacterial food for 15 minutes at the +24 hour timepoint.
(On Wednesday, Tracking Day 2)

55 

After tracking, discard the plates in a biological waste bin.