



Nov 10, 2025

High-throughput Behavioral Assay (HTBA)

DOI

<https://dx.doi.org/10.17504/protocols.io.eq2ly4xzplx9/v1>

Maya Mastronardo¹

¹Johns Hopkins University



Erik Andersen

Johns Hopkins University

Create & collaborate more with a free account

Edit and publish protocols, collaborate in communities, share insights through comments, and track progress with run records.

Create free account

OPEN  ACCESS



DOI: <https://dx.doi.org/10.17504/protocols.io.eq2ly4xzplx9/v1>

Protocol Citation: Maya Mastronardo 2025. High-throughput Behavioral Assay (HTBA). **protocols.io**
<https://dx.doi.org/10.17504/protocols.io.eq2ly4xzplx9/v1>

License: This is an open access protocol distributed under the terms of the **Creative Commons Attribution License**, which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited

Protocol status: Working

We use this protocol and it's working

Created: September 03, 2025



Last Modified: March 02, 2026

Protocol Integer ID: 226371

Keywords: High-throughput behavioral assay, *Caenorhabditis*, loopbio, behavior, video recording, HTBA, *C. elegans*, motif software, nematode strain, amplification of nematode strain, behavioral assay, throughput behavioral assay, *caenorhabditis*, loopbio rig, comparisons across strain, loopbio, strain, consistent developmental staging, embryo collection

Abstract

This protocol outlines a high-throughput behavioral assay (HTBA) for *Caenorhabditis* strains using multiwell NGMA plates imaged on loopbio rigs. The workflow covers preparation of imaging plates, controlled growth and amplification of nematode strains, synchronized embryo collection, and procedures for recording with loopbio's Motif software system.

This approach produces consistent developmental staging and controlled environmental conditions. The expected results are large-scale, standardized video recordings that support comparisons across strains and treatments in genetic and other analyses.

Materials

NGMA (per 1 L)

Pre-autoclave:

- Make in a 2000 mL flask
- add 500 mL of MQ water to the flask
- Add dry ingredients listed below
- add remaining MilliQ water to the flask
- Mix by swirling
- Add tinfoil and autoclave tape to top of flask
- Put in autoclave in autoclave bin with water- Autoclave program 4 - at least 30 min

	A	B
	Peptone	2.5 g
	NaCl	3 g
	Agarose	7 g
	Agar	10 g
	Sterile water	975 mL

Post-autoclave:

- Add the following reagents
- Mix by swirling

	1M KH ₂ PO ₄	25 mL
	Cholesterol (5 mg/ml in EtOH)	1 mL
	1M CaCl ₂	1 mL
	1M MgSO ₄	1 mL

Bleach Solution (Per 200 mL)

40 mL NaOCl (in the cold room, from Fisher, cat #SS290-1), add ~ 100 mL ddH₂O

10 mL of freshly made 10 M NaOH (add 4g NaOH pellets to 10 mL of ddH₂O), add some ddH₂O

Add ddH₂O up to 200 mL



Mix well, store at 4°C (Andersen lab: under the sink in the cold room) until needed. This bleach should be made four days before use. Throw out old bottles of bleach in the fridge as necessary.

3% Polyethylene glycol (PEG) in M9 (Per 500 mL):

Add 15 g of PEG3350 (Catalog # P4338, Millipore sigma) to 500 mL M9. Shake well. Store at room temperature for 15 days.

K medium (Per 500 mL):

51 mM NaCl (**5.1 mL of 5 M NaCl**)

32 mM KCl (**16 mL of 1 M KCl**)

3 mM CaCl₂ (**1.5 mL of 1 M CaCl₂**)

3 mM MgSO₄ (**1.5 mL of 1 M MgSO₄**)

We keep a separate set of salts to only use for HTA experiments. Pour yourself as much as you need into 50 mL conicals, then pipette into a graduated cylinder. Mix the four salts with increasing water, then fill to 500 mL of dH₂O.

Filter sterilize with Thermo Filter Unit (Cat #566-0020).

Add 1.25 µg/mL unfiltered cholesterol (125 µL of 5 mg/mL cholesterol) after filtering.

Mix, label, and store for up to two weeks. Be sure to check the K medium prior to use for any floating particulate, which is a sign of contamination. If K medium is contaminated, pour it down the drain and make a new K medium. Record which batch you used of each reagent (date/initials) in your lab notebook; this way we can quickly figure out if a batch of something has gone bad.

Frozen HB101 bacteria:

1. Prepare enough HB101 for all planned experiments for an entire project at one time.
2. HB101 will be frozen at OD100 in aliquots of appropriate sizes (1, 5, 10, 15 mL, etc.)
3. Thaw enough HB101 for the day, depending on your need. Using equal amounts from different batches reduces potential variation associated with differences in batch.
4. Dilute OD100 HB101 with K medium to the appropriate concentration
5. Dilute 1:3.333 to OD 30
6. Add kanamycin (18.75 µL of 80 mM kanamycin per 10 mL of solution).
7. Vortex to resuspend before pipetting into wells.

Plate preparation (Multiwell plates - 1 week before imaging):

- 1 Make 1 L NGMA for pouring both 96-, 24-, or 6-well plates. Because you are autoclaving the NGMA, autoclave a bottle of MilliQ water. This sterile water will be used to keep the WellJet dispensing the cassette warm during plate pouring. If you are pouring a few plates (fewer than 10), you should prepare a minimum of 500 mL of NGMA.
- 2 For N2, we grow worms for 67 hours after feeding to get adults (65 hours for C. briggsae). Gently pipette 15 μ L of worm culture (~10 worms +/- 5 worms) from the multiwell plates to each well of the previously prepared multiwell plate using a 5 - 50 μ L 12x multi-channel pipette. After adding worms, gently tap the plate from side to side to get the worms off the sides and into the agar.
- 3 Incubate the plates at room temperature in the hood in the plate pouring room for 60 minutes before imaging to dry the liquid after pipetting the worm solution. Turn on the fan in the hood and rotate the plate after 30 minutes to ensure even drying. Confirm that all liquid has been absorbed before recording the plates.
- 4 Make sure the water bath is turned on and set to  60 °C . Remove the NGMA from the autoclave and allow it to sit for 10-20 minutes before adding the post autoclave salts. Place the flask with the NGMA into the water bath.
- 5 Make 1 L NGMA (recipe at end) for pouring both 96-, 24-, or 6-well plates. Because you are autoclaving the NGMA, autoclave a bottle of MilliQ water. This sterile water will be used to keep the WellJet dispensing the cassette warm during plate pouring. If you are pouring a few plates (fewer than 10), you should prepare a minimum of 500 mL of NGMA.
- 6 Make sure the water bath is turned on and set  60 °C . Remove the NGMA from the autoclave and allow it to sit for 10-20 minutes before adding the post autoclave salts. Place the flask with the NGMA into the water bath.
- 7 Ensure the waste beaker is properly placed in the tubing.
- 8 Open a new package of multiwell plates (96-well: Cytvia Cat. No. 7701-1651, Universal Lid Cytvia Cat. No. 09-003-44, 24-well: ThermoFisher Cat. No. 142485, 6-well: Corning Cat. No. 08-772-1b). Label each plate according to the planned assay.
- 9 Turn on the WellJet. If a plate is present on the platform from previous use, remove it. Press OK to allow the device to initialize.



- 10 Load the 4x EasySnap, 8-channel cassette, 5-9'999 μL (Integra part no. 5101) labeled for NGMA dispensing.
- 11 Place the tubing in the autoclaved water and prime 3-5 times to warm the tubing. Recover the liquid two times to ensure that no residual water remains in the tubing.
- 12 Transfer the tubing into the flask containing NGMA.
- 13 Select the "Loopbio 96-well Plate Full NGMA" (or other appropriate multiwell NGMA program). Load the multiwell plate onto the loading dock with the A1 position on the multiwell plate matching the A1 written on the WellJet loading dock.

NGMA volume per plate layout:

	6-well	4 mL
	24-well	1 mL
	96-well	200 μL

- 14 Prime the cassette by pressing PRIME. This step will dispense some liquid into the waste trough so all the liquid dispensing will be consistent. Prime the cassette twice before using to be sure agar reaches the nozzles and dispensing is even across all channels. Confirm that liquid is being pulled through each tube that all dispensers are ejecting agar uniformly by visual inspection.
- 15 Press RUN to dispense the NGMA into the multiwell plate.
- 16 After dispensing, remove the multiwell plate. Keep the tubing in the flask and continue filling as many plates as desired. For each additional multiwell plate, prime once before dispensing into the new plate.
- 17 When finished, run the cleaning program for the cassette to eject NGMA and wash the cassette. The program will instruct changing of solutions for cleaning. You should prepare MilliQ water and 70% ethanol if the stock solutions near the WellJet are empty.
- 18 Discard the waste in the beaker. Place the beaker into the normal lab dish washing area. Clean the waste trough and tubing using soap and water. Then, rinse with MilliQ water.



- 19 Remove cassette and power off the WellJet.
- 20 Once all plates are poured, allow them to cool for 20-30 minutes next to the WellJet station. Once the agar is solid, stack plates and place a clean lid on top.
- 21 Weigh each individual plate (uncovered with no lid) and record the weight.

Note

Avoid using plates with a mass 2% above or below average. For example, if your poured plates (NGMA + plate, lid off) weigh 50 grams on average, avoid using plates that weigh below 49 grams or above 51 grams.

Plate drying:

- 22 Place the cooled and weighed 96-well NGMA plates (uncovered) in an empty cardboard box. Arrange the plates in a single layer and do not stack the plates. Six plates can fit into each cardboard box.
- 23 Place the cardboard box in the oven set to  65 °C and dry for one hour.
- 24 Remove the plates, weigh again, and record the weight. The goal is to reach a mass loss of 3 to 5% after drying.

Note

Once dried, plates can be stored for up to one month upside-down at  4 °C in a sealed box to reduce further drying.

Worm preparation:

25

Note

- Store all source plates at  15 °C . The source plates used for chunking should be **no more than one month old**. If using a newly thawed strain, allow the plate to starve and then chunk from the new source plate.
- For picking each generation, use plates from the **same batch** of NGMA and OP50. Before starting, make sure that you have enough supplies for each generation.
- For loopbio experiments, aim for 10 worms per well in the final imaging plates. To achieve this number, we titer cultures so that each growth well contains approximately 50 worms, which allows pipetting an appropriate volume to yield ~10 worms per imaging well after final growth. Depending on how many wells per strain your experiment requires, you should amplify more animals than the final number to account for losses during washing and filtering. Estimate how many worms or plates to prepare by working backwards from the number of final wells required and multiply the number of wells by 10 (worms per well) and scale up accordingly to ensure sufficient material at each step.
- When possible, label plates using **printed labels** (avoid handwritten strain names).
- **Label all plates and tubes** before the steps that require them.
- Organize plates containing the same strain into the same box when possible (**do not stack different strains together**). Whenever possible, organize groups of strains into boxes such that easily confusable strain names are not in the same box. Also, mark plates with confusing names so that people will more easily notice the differences
- Monitor the temperature in all incubators to make sure that they match the temperature in the protocol using an iButton.

- 26 Using a sterile, autoclaved spatula, chunk from a starved plate onto two fresh 6 cm plates. Store the chunked plates at  20 °C .
- 27 After 48 hours, pipette 15 µL of bleach solution away from the lawn on a new plate and pick ten to twenty gravid hermaphrodites into the bleach spot. Swirl gently with the pick and store plates at  20 °C .
- 28 After 24 hours, pick 20-30 L1s to a new labeled 6 cm plate for each strain. If the L1s did not survive the bleach, make a note of it so that larger chunks or fresher source plates can be used for future assays. This pick is generation zero (G0). Store these 6 cm plates in the  20 °C incubator.
- 29 After 48 hours, pick five late-stage L4 hermaphrodites to labeled 6 cm plates for each strain. This pick is generation one (G1).

Note

Worms might have grown into young adults by this time. In which case, pick the youngest adult worms that you can find. If you find males on source plates, avoid picking young adults from these plates. Check the plates one and two days after picking to make sure that they will be properly staged for the generation two pick. Grow this generation of animals at 21.5°C.

- 30 Make bleach for conical bleach and filtration on the day of your G1 L4 pick. Also, make K medium (recipes at end).
- 31 Four days later, pour ~5 mL of M9 onto one plate, transfer the liquid to the next plate and then transfer to a 15 mL conical tube, repeat in the reverse direction for a second "wash" of all plates, transfer to the same 15 mL conical.
- 32 Spin down worms at 254 g (Andersen lab: Eppendorf 5810R, 1100 rpm) for 1 minute using the table-top clinical centrifuge.
- 33 Aspirate off the M9 (be conservative and leave ~2 ml M9 to prevent aspiration of worms).
- 34 Add 6 mL of bleaching solution to each 15 mL conical.

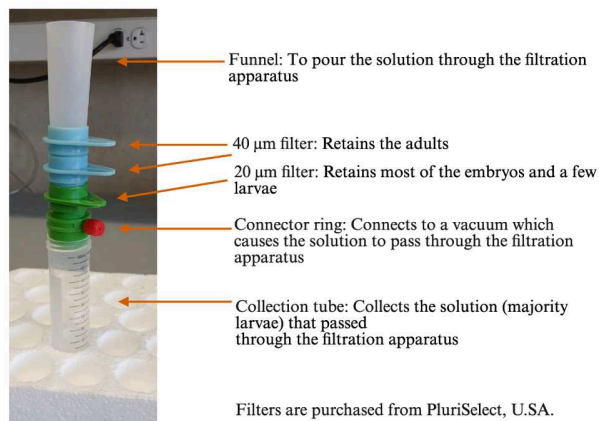
Note

The freshness of the bleach matters.

- 35 Shake the tube manually and vigorously for four minutes. This step can take as little as three minutes. Be very careful not to over-bleach the worms. Once you see that the carcasses are nearly dissolved, move to the next step. If only embryos are left, then you have gone too long.
- 36 Once you find that only a few adults remain, move quickly to spin down the embryos at 254 g (1100 rpm) for 30 seconds. It is best to have the reagents next to the waste/pour-off receptacle and prepared for the next few steps. If you feel that you have under-bleached your worms, you can let them spin down for a full minute.
- 37 Immediately decant the bleach into a waste receptacle carefully, in one smooth motion (no shaking), without disturbing the worm pellet.



- 38 Immediately pour 10 mL of M9 into each 15 mL conical tube (have 50 mL conicals filled with M9 at the ready for each wash).
- 39 Invert several times to make sure the pellet is resuspended (as you walk back to the centrifuge).
- 40 Spin down the embryos at 254 g (Andersen lab: Eppendorf 5810R, 1100 rpm) for 30 seconds.
- 41 Decant the M9 into a waste container carefully and quickly (one smooth motion) without disturbing the worm pellet.
- 42 Repeat steps 12 - 16 twice with M9 spinning for 1 minute.
- 43 Do final resuspension in 3 mL of M9.
- 44 Determine the titer of the embryos by counting the number of embryos in five replicates of 3 μ L droplets and take the average. Be sure to vortex the conical well before pipetting three 3 μ L droplets at a time. When titering, if you see more than 50 embryos per droplet, add more M9 to the conical tube to get an accurate count of the concentration of embryos. If your droplets vary by +/- 10 embryos, repeat and count more droplets.
- 45 Use this titer amount to make 6 cm plates with 450 embryos on each plate. Leave at  21.5 °C for three days.
- 46 After three days, set up the filtration apparatus. A connector ring is attached on top of a clean and labeled 50 mL conical tube. Above the connector ring, a filter stack consisting of a 20 μ m filter (green) at the bottom and two 40 μ m filters (blue) at the top are added. A funnel is attached to the 40 μ m filter to pour the solution through the filtration apparatus. (pluriStrainer® 40 μ m: PluriSelect Cat #: SKU 43-50040, pluriStrainer® 20 μ m: PluriSelect Cat #: SKU 43-50020, Connector Ring: PluriSelect Cat #: SKU 41-50000, Funnel: PluriSelect Cat # SKU 42-50000)



Filtration apparatus set up.

- 47 Add 2 mL of M9 to each plate. Swirl and discard the liquid. Repeat the process one more time. This step is to get rid of the adults and larvae that will not be stuck to OP50. You will NOT be keeping these worms.
- 48 Next, add 2 mL of M9 to each plate. Take a rubber policeman (Fisher Scientific Cat # S66001) and gently scrub the plate to remove the embryos. Collect the solution in a 15 mL tube. Repeat the process one more time. This solution (embryo solution) will be used for filtering through the filtration apparatus.
- 49 Mix the embryo collection by inverting the 15 mL tube four to five times to ensure that the embryos are in the solution and not settled at the bottom of the tube.
- 50 Pour the embryo solution through the funnel of the filtration apparatus.
- 51 Connect the connector ring to the vacuum. Start the vacuum to pass the embryo solution through the filtration apparatus. Make sure that the solution is collected in the 50 mL conical tube. The vacuum should only be applied for 1-2 seconds, which is enough time for the solution to pass through the filter.
- 52 Remove the funnel (clear tube) and the two 40 µm filters (blue).

- 53 To collect the embryos retained on the 20 μ m filter (green), reverse the direction of the 20 μ m filter (green) and place it on top of a clean and labeled 50 mL conical tube. Make sure the top of the filter is facing the inside of the 50 mL tube.
- 54 Using a serological pipette, add 4 mL of 25% bleach solution to the reversed 20 μ m green filter.
- 55 Once all the solution has passed through the 20 μ m green filter, start the timer for 30 seconds. Keep swirling the tube during this incubation time. Ideally, only the embryos should be recovered from this step. However, the purity of the embryos is approximately 94 - 98% (based on data from at least 20 independent batches of filtrations). The lack of purity is because a few larvae are stuck to the 20 μ m green filter along with the embryos and are collected with the embryos. Adding 25% bleach solution will kill the larvae, which is important because we want no larval contamination to have staged populations for our high-throughput assays.
- 56 After 30 seconds, add 6 mL 3% polyethylene glycol (PEG) in M9 (w/v) on top of the 20 μ m green filter to make up the volume to 10 mL. This step will allow for any embryos that are still stuck on the 20 μ m green filter to be removed and collected in the 50 mL collection tube. Transfer the contents to a clean and labeled 15 mL conical tube and centrifuge at 3,197 g (Andersen lab: Eppendorf 5810R, 3,900 rpm) for one minute to pellet the embryos.
- 57 Discard the supernatant and add 10 mL of 3% PEG in M9. Centrifuge at 3,197 g (3,900 rpm) for one minute. Repeat the step once more.
- 58 Discard the supernatant and add 10 mL of 3% PEG in K medium. Centrifuge at 3,197 g (3,900 rpm) for one minute.
- 59 Discard the supernatant and add 3 mL of K medium.
- 60 Titer embryos and dilute your yield to be 1 embryo per μ L.
- 61 Pulse vortex each 15 mL conical of 1 embryo per μ L for at least ten seconds before pouring into the reservoir (if you are pipetting one worm strain per row, if not pipette directly into the wells).
- 62 Pour into the reservoir and mix before pipetting into assay growth plates. For mixing, take 50 μ L from the bottom of the trough and dispense it higher up on the side of the reservoir. Repeat on the other side for a total of ten times (five per side). This step is critical to ensure proper embryo dispensing and titers.

- 63 Pipette 50 μL of embryo solution to distribute approximately 50 animals into each well of the tissue-culture treated 96-well flat-bottom plate (USA Scientific 96 well Cellstar Clear TC PLT*FLAT Cat #: 665-180).
- 64 Remember to mix each well or trough five times before aspirating and dispensing to tissue-culture multiwell plates. We use 12-channel reservoirs (USA Scientific 12-well V-Bottom Reservoir Cat #: 3824*3412) if pipetting a strain per row. If you are using a single strain per well, then you can pipette directly into the 96-well growth plate or create a master plate (Sterile, Flat Bottom with Lid, Individually Wrapped, 100 per Case, Cat #: 141380).
- 65 Seal all 96-well plates with a gas permeable sealing film (Axygen BF-400 gas permeable sealing film: Fisher Cat #: 14-222-043). Try to avoid wrinkles in the film by using a roller and running your finger nail along the outer wells.
- 66 Shake overnight at 170 rpm at $20\text{ }^{\circ}\text{C}$ in a freshly made humidity chamber. To make a humidity chamber, place damp paper towels in a clean (bleached and rinsed) plastic box (IRIS USA CNL clear Latching Box, 6 Qt, 18 Count; or similar box). After filling the humidity chamber with plates, close the lid and seal the chamber using parafilm. These chambers hold 18 plates.
- 67 The next day, check that all animals have hatched into swimming L1 larvae. If the worms did not hatch, start over.
- 68 Dilute frozen and previously prepared OD100 HB101 food (see protocol for preparation) to a concentration of OD30 with K medium. Add kanamycin (US Biological, Cat #: K0010) to the OD30 food mix. The final kanamycin concentration in the well should be 50 μM (final concentration in the food dilution, prior to plating, will be 150 μM). That is 18.75 μL of 80 mM stock per 10 mL of solution. Make sure to resuspend the bacteria before you add it to the plates by swirling (if a large volume of OD30 bacteria is made in an autoclaved Pyrex media storage bottle) or vortexing (if using smaller batches of OD30 bacteria made up in a conical tube or otherwise vortexable container).
- 69 Add prepared and vortexed food to a reservoir. Pipet 25 μL of food from the reservoir to each row of growth plate with arrested L1s. Feed one imaging plate on average every 10-15 minutes (This step can vary depending on what imaging program that you plan to run). Use a timer to make sure that you do not forget. Ensure that the bacterial food is mixed into each well. Every well should look cloudy. It is easiest to see when you look at the wells above a black background. If you are running many plates, start making food in the morning.
- 70 Seal with a gas permeable sealing film (Axygen BF-400 gas permeable sealing film: Fisher Cat #: 14-222-043).



- 71 Label the plate top with the plate number and any condition information, and record the time that you added the food solution.
- 72 Put the plates back in the humidity chamber once they are fed. When done for the day, make sure your paper towels are still damp and parafilm the chamber shut.
- 73 Return the humidity chamber to the incubator and shake at 170 rpm at 20°C for 67 hours (You can use this time calculator to time the feedings). Stagger your feedings in the order that you will image the plates (transferring worms takes ~5-7 minutes, drying takes ~60 minutes, and imaging takes a variable amount of time depending on the program that you choose to run).

Seed and drug plates (the day before imaging):

- 74 Remove the imaging plates that you plan to use from the cold room and let them warm to room temperature before adding drugs or seeding.
- 75 Prepare a media bottle or tubes of DMSO (control) or drug. If you are using multiple drugs or concentrations, each tube of the cassette can be placed in different microcentrifuge tubes, meaning each row will be a different drug/concentration so plan your plate layout accordingly.

Note

Prepare drug stocks that are 100X your desired test concentration. The final concentration of DMSO in the media must be 1%, so your compound will be diluted 1:100 when it is added to the plate. If you plan on adding different concentrations of a drug in a single plate, note that the WellJet can dispense different concentrations or compounds in each row of a multiwell plate (96-well plate = 8, 24-well plate = 4, 6-well plate = 2). You cannot separate by column.

- 76 Place a DMSO/Drug cassette (a labeled 4x Easy Snap, 8 channel Cassette, 0.5-500 μ L - Integra part no. 5100) into the WellJet.

Note

If you are using multiple compounds or concentrations, separate the tubing and place one in each conical tube. Make sure that the tubing is ordered correctly to reflect your plate design.



- 77 Prime the tubes and run the “Drug” program for the appropriate multiwell plate to dispense DMSO/Drug to each well of the multiwell plate. You do not need to prime in between plates.

Note

If you are switching between drugs diluted in DMSO, clean with DMSO, then 70% ethanol, DMSO (this is not a cleaning program just prime a few times with each before you switch to the new cassette).

Volume of Drug/DMSA based on plate layout:

	6-well	40 μ L
	24-well	10 μ L
	96-well	2 μ L

- 78 Allow 30 minutes to an hour for the liquid to become absorbed into the agar, on the benchtop next to the WellJet. Immediately start seeding your plates.
- 79 Clean the Drug/DMSO cassette. If you use just DMSO for control, you can immediately clean with the Extra air cleaning protocol. If you used any drug, run the “switching between drug protocol” noted above and then end with the Extra air cleaning program.
- 80 Calculate the amount of OP50 needed and multiply by 1.5 because some bacteria solution will be lost in priming. Place an aliquot of OP50 solution. Make sure to keep mixing by swirling.
- 81 Place an OP50 cassette into the WellJet.
- 82 Prime the tubes and run the “OP50” program for the appropriate multiwell plate to dispense the correct proportion of OP50 to each well of the multiwell plate. You do not need to prime in between plates because you are not transferring tubing but intermittently swirl the OP50 to keep well mixed.

Volume of OP50 based on plate layout:

	6-well	152 μ L
	24-well	30 μ L

	96-well 10 μ L

- 83 Allow 30 minutes to an hour for liquid to become absorbed into the agar, on the benchtop next to the WellJet. Place a lid on the plates and stack upside-down in a plastic container (same type as the humidity chamber).
- 84 Run the OP50 Cleaning program to clean the cassette. The program begins with two washes labeled "Deionized Water." Replace the second DI water wash with 10% bleach to ensure that any residual bacteria is removed from the tubing.
- 85 Leave the plates at room temperature overnight.

Note

I tend to leave the plates in Levi 32 overnight because it is where the loopbio rigs are and it is cooler.

Transferring worms to plates:

- 86 For N2, we grow worms for 67 hours after feeding to get adults (65 hours for C. briggsae). Gently pipette 15 μ L of worm culture (~10 worms +/- 5 worms) from the multiwell plates to each well of the previously prepared multiwell plate using a 5 - 50 μ L 12x multi-channel pipette. After adding worms, gently tap the plate from side to side to get the worms off the sides and into the agar.
- 87 Incubate the plates at room temperature in the hood in the plate pouring room for 60 minutes before imaging to dry the liquid after pipetting the worm solution. Turn on the fan in the hood and rotate the plate after 30 minutes to ensure even drying. Confirm that all liquid has been absorbed before recording the plates.

Recording worm behavior:

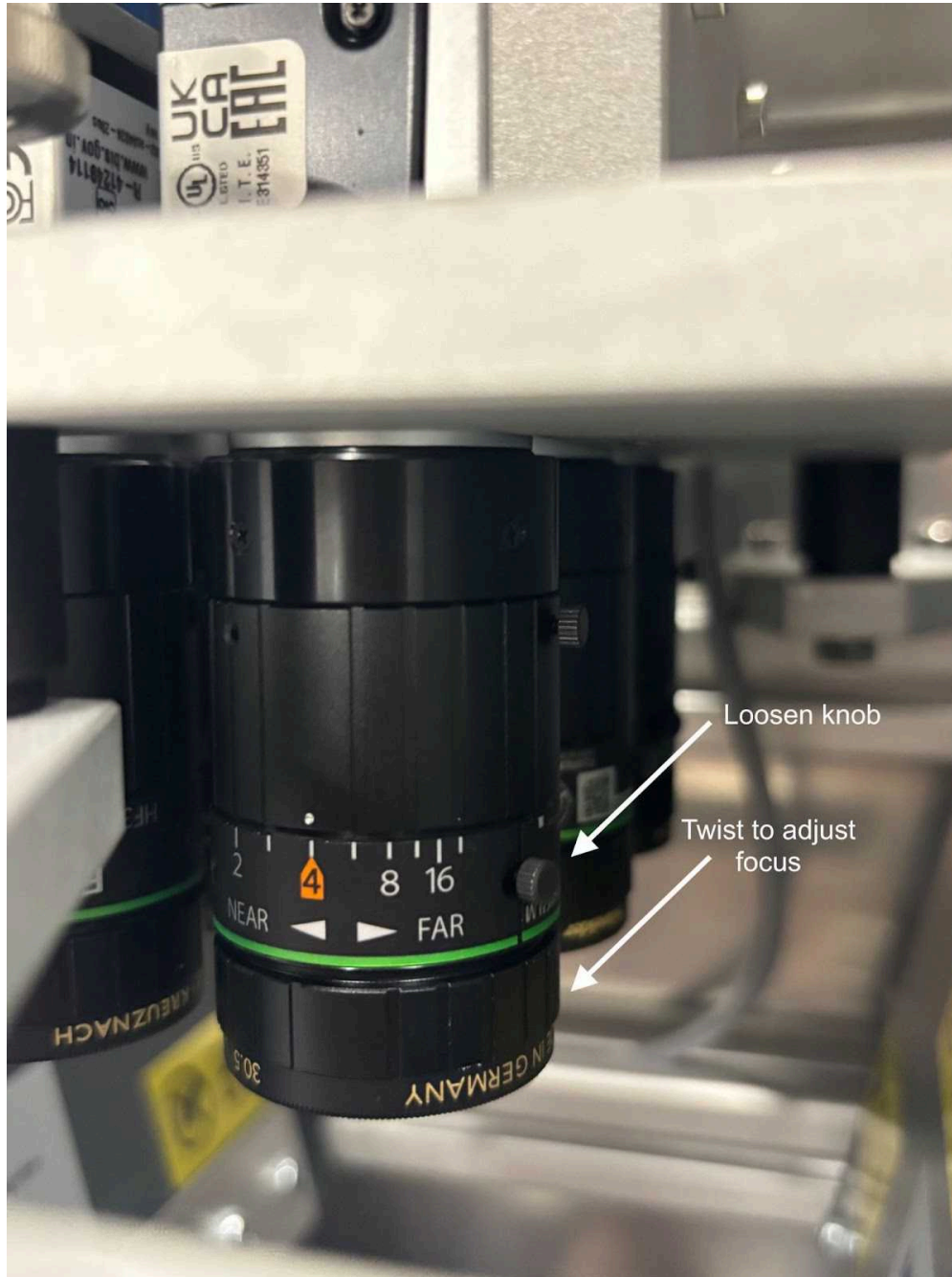
- 88 Turn on the computers (button on back, top middle), loopbio light boxes (switch on bottom left), and fans on top of each rig (the power strip behind the monitor). Wait for the login screen to appear. Select "lab" and type "lab" as password.
- 89 Click on the Motif (looks like a blue and white "M") on the top right of the screen to open the Motif imaging software.



- 90 Once Motif is open, you will see a list of numbers on the left of the screen representing the high-resolution cameras (Local Cameras) available for imaging. Click “Connect” for all cameras that you want to use (you can use all six cameras in parallel). Wait until the camera serial number (e.g. 22075785) changes to blue and the camera and preview image is visible in “Connected Cameras”.

Note

Cameras can be manually focused and should be checked every three months. To adjust the focus, open Motif and connect to the cameras. Use a plate with worms to help assess focus. Identify any cameras that appear out of focus, you can cover each lens with your hand to determine which image corresponds to which camera. Once identified, loosen the lower of the two small knobs and manually twist the lens to adjust focus. Repeat this process for all rigs and cameras.



- 91 Open the door of the loopbio rig and insert the plate with worms on it (it can only take one plate at a time). The plate fits into a recess on the platform. Push the plate as far back as possible in the recess. Make sure that all of the plates are inserted so that well A1 is in the bottom left. Leave the plate lid on to provide some protection from the bluelight stimulation. If the plate lid is a bit foggy, wipe the lid and bottom of the plate with a Kimwipe sprayed with 70% ethanol.

- 92 Close the door.
- 93 Navigate to the I/O & Scheduling tab. This step will allow you to adjust IR and blue light intensity.
- 94 Enter the value 5.5 into the field for IR LED (if not already set). Change this value for adjusting the brightness of all cameras simultaneously (larger value = brighter, lower value = darker). Scroll through the cameras to see if the wells look in frame.

The screenshot displays the 'I/O/Scheduled' tab of a control interface. It features two sliders under the 'Outputs' section: 'BLUE_LED' is at 0.0 and 'IR_LED' is at 5.0. The 'Sensors' section provides real-time data: LUX is 0.0003, Humidity (inside, relativ humidity) is 20.63, Temperature (inside, deg. Celsius) is 29.02, and Blue_LED State (should be <4) is 0.0. A 'Scheduled Operations' box shows a timestamp '2022-01-10T16:59:11' and the message '... no scheduled tasks ...'. Below this are 'Show', 'Simulate', and 'Clear' buttons. The 'Experiment Scripts' section at the bottom has a dropdown menu with 'set_cameras_to_10fps.py' selected and 'Stop' and 'Start' buttons.

- 95 To start recording, open the terminal and run `run_6minBluelight_3_10spulses_withPrePost.py` or whichever recording program that you wish to run.
- 96 For transferring video files, you will need to copy the files in /mnt to a hard drive or HPC. This step will take a while because the movies are large.



Note

For the newer loopbios have two storage directories in /DATA1 and /DATA2 in /mnt. You will need to copy over the videos from each storage directory because each holds data for three out of the six cameras.