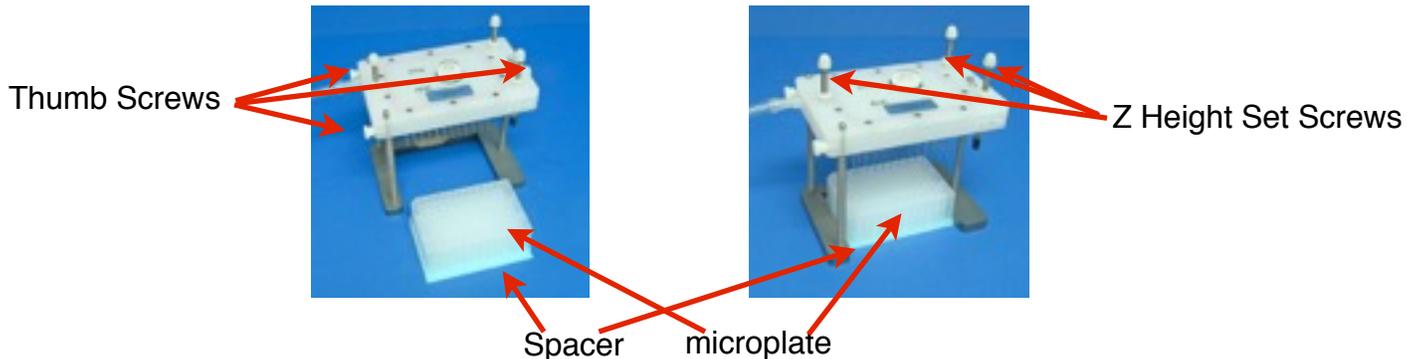


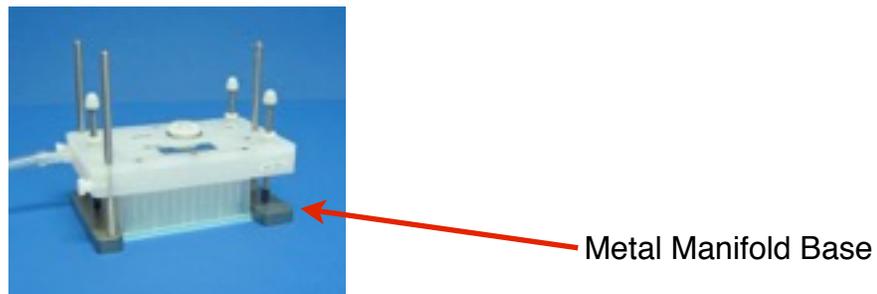
Bleaching and Dispensing of *C. elegans* using the BioTek MicroFlo Select

Bleaching

1. Autoclave the cassette for the BioTek MicroFlo Select by wrapping in foil and autoclaving on dry cycle for 10 minutes.
2. Set up the 96-well aspirator using an appropriate spacer.
 - You want the aspiration tubes of the aspirator to go down far enough such that ~100 μ l remains after aspirating.
 - For Arcitc White Plates (cat # AWLS-X1005), using three c-fold paper towels (cut so they fit under the aspirator) works well.
 - To set up the 96-well aspirator:
 - i. Place the spacer under a microplate. Slide the spacer and microplate, under the VP 177A-1 as in Figure 3b. Note: the spacer can be any height depending on how far into the wells the aspiration tubes need to go.



- ii. Make sure that the three Z height set screws are not set longer than the aspiration tubes.
- iii. Loosen the three thumb screws and slide the manifold down until the tubes rest on the bottom of the wells of the microplate as in Figure 3c.



- iv. Tighten the thumb screws to lock the manifold into place. Use the provided bubble level to ascertain that the manifold is level.
- v. While in this down position, move the set screws so that the bottom of each screw touches the metal manifold base. Check the bubble level again and make adjustments if necessary. Lock the set screws into position by turning the lock nut on each screw so that they contact the top of the manifold.
- vi. Remove the microplate and spacer. Add one ml of water to each well to test how much liquid remains after aspiration.

- b. Prepare any balances you might need during the experiment if you are doing an odd number of plates. (e.g. If you plan to add 700 μ l of bleach to each well, you need to make a balance plate that has 800 μ l of water per well to include the 100 μ l left behind after aspirating).
 - This is especially important when you need to spin down while the worms are in bleach. You do not want to waste time while bleaching.
3. Use 2 ml of M9 to wash worms off from plates and add to the appropriate well. Add the M9 to the first plate, gently shake the M9 back and forth in the plate, dump the M9 onto the next plate and shake the M9 back and forth. Continue this for each plate needed for one well. After the final plate, transfer the worms using a Pasteur pipette.
 - It is necessary to add the same volume to each well so that the 96-well aspirator functions properly.
4. Spin down the worms at 1100 rpm for 1 min using the 5810R clinical centrifuge .
5. Remove the M9 using the 96-well aspirator ((VP 177A-1, V&P Scientific).
 - ***When using the aspirator be aware that the aspirator will leak after aspiration! After you finish any aspirating, gently push or slide the plate out of the manifold base. Once the plate is clear of the aspirator, hold a paper towel gently against the aspirator pins and tilt the aspirator towards the side with the tubing to clear any liquid remaining in the manifold. Gently blot the pins to remove any remaining moisture.***
6. Add 700 μ l of bleach solution using the BioTek MicroFlo Select.
7. Shake on the microplate vortexer at 1450 rpm for 4 min.
 - You might have to put paper towels in the vortexer to help keep the plate snugly in place.
 - The time on the shaker will depend on the freshness of the bleach. If the bleach solution is fresh, you might want to shake for less time at first.
8. While the plate is shaking, run 2 ml of distilled water through the BioTek MicroFlo Select cassette. This will remove traces of bleach.
9. After running distilled water through the cassette, prime the cassette with M9 for the washes.
10. Spin down the worms at 1100 rpm for 1 min using the 5810R clinical centrifuge.
 - **Always make sure to remove the sealing mat carefully to avoid cross contamination.**
11. Check the whether worms are dissolved by using a Pasteur pipette to suck the material out of a one of the wells and look at the worms under the microscope.
 - How quickly the worms dissolve will be dependent on the freshness of the bleach solution and the bleach itself.
12. Return the material from the “bleach test” well back to that well.
13. If the worms are not dissolved, repeat steps 7-11 (but only shake for **1 minute** each time) until the worms are dissolved.

14. If the worms are dissolved, spin down the worms at 1100 rpm for 1 min using the 5810R clinical centrifuge.
15. Use the 96-well aspirator to remove the bleach.
16. Use the Biotek MicroFlo Select to add 700 μ l of M9 with the "1 mL Wash" program.
 - Don't place the tubing head directly in a large, communal bottle of solution. Always decant the M9 you want to use in a separate bottle so that you do not contaminate the larger bottler.
17. Check to see if the worms are well resuspended. If not, resuspend the worms by shaking on the multiplate vortexer for 10 seconds.
18. Spin down the worms at 1100 rpm for 1 min using the 5810R clinical centrifuge.
19. Remove the M9 using the 96-well aspirator.
20. Repeat steps 16-19 two more times for a total of three M9 washes.
21. During your last spin, remove the M9 from the BioTek MicroFlo Select cassette and prime the cassette with S Medium.
22. Add 400 μ l of S Medium.
 - Don't place the tubing head directly in a large, communal bottle of solution. Always decant the M9 you want to use in a separate bottle so that you do not contaminate the larger bottler.
23. Gently resuspend the worms and transfer 50 μ l to a Costar Corning 96-well culture plate.
 - Make sure to resuspend the worms in a single well between **each** addition of 50 μ l to a culture plate.
 - This should give you about 100-200 embryos per well.