# Worm Array* loading protocol

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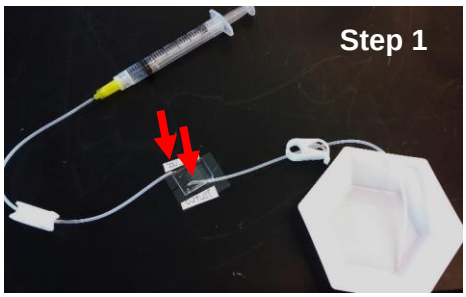
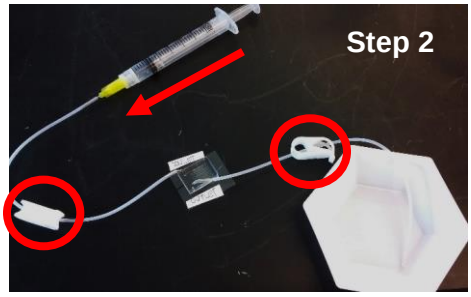
Preparation:

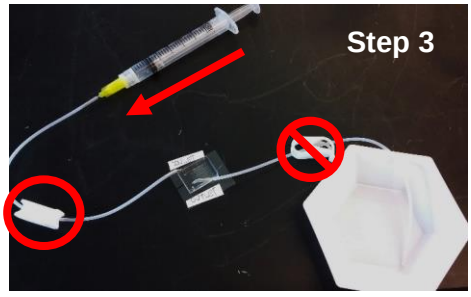
1. Synchronize L4 populations (Need ~ over 100 per device)
    a. Bleach large plate of gravid adults and wait ~48-60hrs depending on growth rate
2. Make array devices using soft lithography techniques
3. Prepare 3 syringe tubes with pin and silicone tubing for:
    a. Degassing (load with buffer)
    b. Worms
    c. Immobilization agent (load with tetramisole hydrochloride~100mM)
4. Prepare 1 tube with pin for outlet

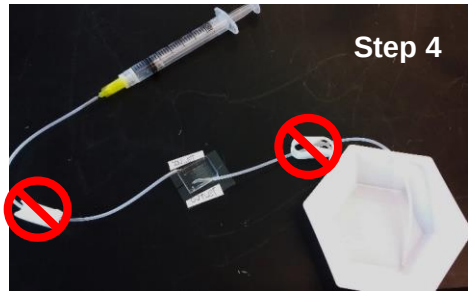
Setup and degassing:

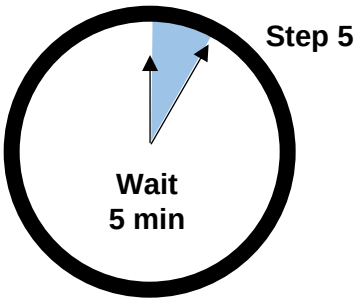
1. With pinch clips already on tubing, insert pins to the device with the syringe being connected to the inlet
2. Open clips and manually flow buffer through device using degassing syringe
3. Pinch the outlet clip to close the flow and gently push the degassing syringe
4. While pressurizing the device by pushing the syringe, close the inlet tubing with the pinch clips
5. Let device degas for 5-10minutes – until no bubbles are seen in device
6. Open both clips

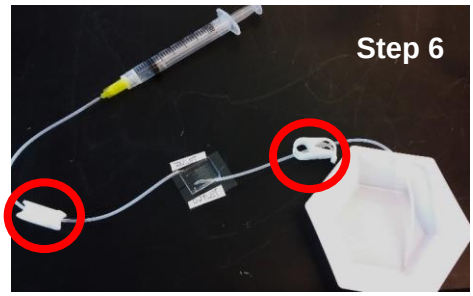


Worm loading:

7. Wash a dense worm plate and withdraw liquid into syringe to a volume of ~1mL
8. Switch degassing syringe to worm loading syringe without introducing bubbles in the pin. *Make sure no bubbles are present by allowing some liquid from syringe to overflow.*
9. Set up loading syringe vertically to allow for worms begin to settle with gravity (this prevents them from settling on the side of the syringe if held horizontally), and unclip pinch clips and remove outlet pinch clip
10. Flow between 2.5-5ml/hour to load animals
11. Observe worm loading under the microscope.
12. Once done, clip the **inlet** valve. (Outlet clip should be off but if it is still there do not touch or disturb outlet valve and take off clip)
13. Switch loading syringe to immobilization syringe
14. Unclip inlet valve to load at similar flow rate for up to 5 minutes, or until worms have stopped moving
15. Once worms are immobilized, clip **inlet** valve
16. You can maneuver the device as long as only the inlet pinch is clipped
17. Image worms under high or low magnification



* See Reference:
Hyewon Lee, et. al., “A multi-channel device for high-density target-selective stimulation and long-term monitoring of cells and subcellular features in *C. elegans*”, **Lab on a Chip,** 2014,14, 4513-4522. DOI: 10.1039/C4LC00789A