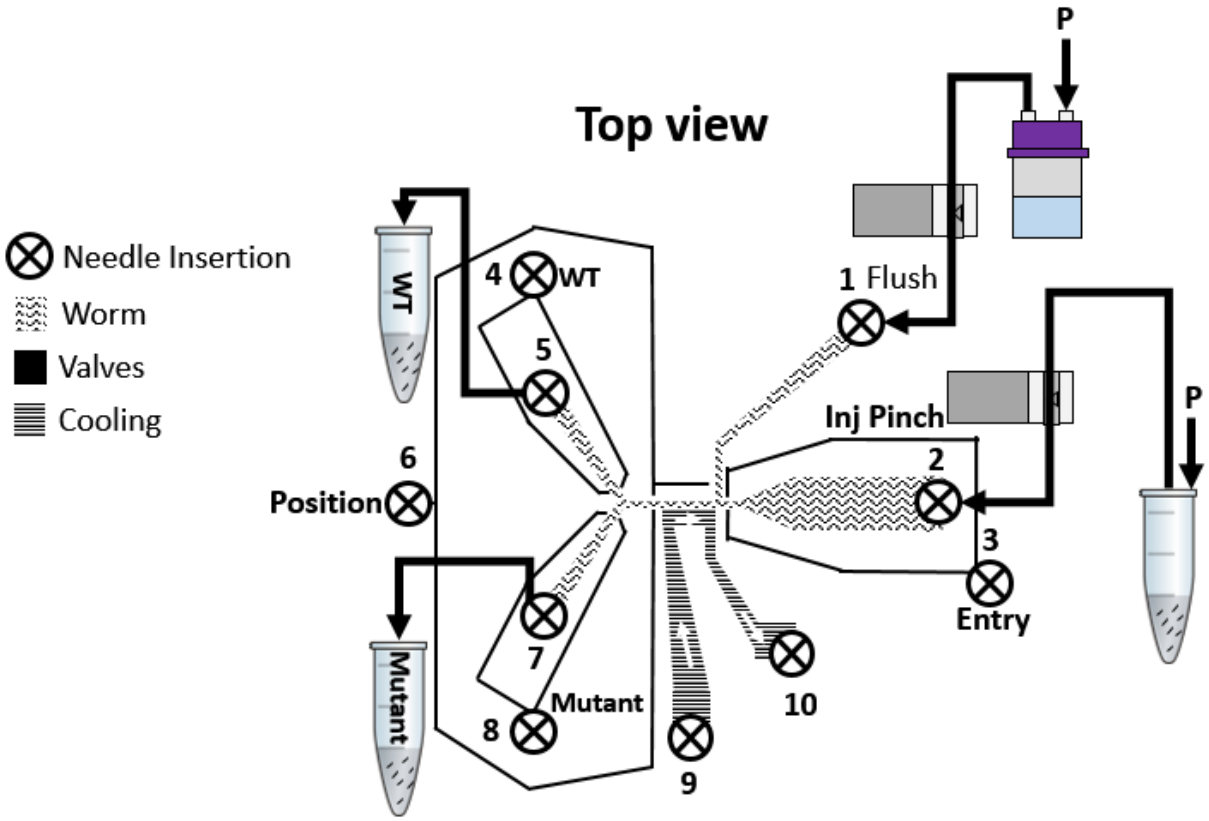
Sorting Device Overview:



Tubing Connections: (see diagram for numbered tubing locations)

On-chip valve tubing #s: 3, 4, 6, 8

- Entry valve (3)
- Position valve (6)
- Wild-type outlet valve (4)
- Mutant outlet valve (8)

Flow tubing #s: 1, 2, 5, 7 → remember to clean these thoroughly to avoid future clogs

- Flush channel (1)
- Inlet channel (2)
- Wild-type outlet channel (5)
- Mutant outlet channel (7)

Cooling tubing #s: 9, 10 → not used in SOP; can potentially be used by flowing cooling liquid for immobilization

Failure Modes for Sorting Devices – Troubleshooting

Devices failing is frustrating, especially when they are being used with very expensive microscopy equipment. We suggest you read this guide before attempting to set up devices and **practice on a dissecting microscope** before upping the ante.

Here are some common issues and what you can do to alleviate them:

1. **Fluid leaks out of device at PDMS-air interface**
    - **a.** Often due to poor hole-punching, leading to small cracks in the PDMS surface. Make sure your syringe tip or biopsy punch is sharp and that you are removing these from the PDMS gently when punching holes through PDMS during fabrication.
    - **b.** You may need to use a different gauge syringe tip or biopsy punch. If the needle does not create a tight seal in the hole, leaks like this will occur.
    - **c.** May be due to poor insertion of metal syringe tip into PDMS when connecting tubing. Particularly if the hole is punched at an angle and the syringe tip is not inserted following that angle, it is easy to create microcracks by inserting the syringe tip straight down. These cracks, even those internal to the PDMS, can eventually lead to leaks over the course of an hour or more.
2. **Fluid leaks out of device at PDMS-coverslip interface**
    - **a.** This is almost exclusively due to poor plasma bonding at the PDMS-coverslip interface. Adjust the time you leave the plasma on to tune the amount of surface activation, which will in turn adjust bonding strength. We also suggest that when initially de-gasing on-chip single layer valves, you keep pressures relatively low, between 15-20psi. Once de-gasing is complete, gradually increase the pressure until the single layer valves close sufficiently. Of all the types of failures, these are the most explosive, so use caution when operating at high pressures and set-up and test fluidic components as best you can before mounting the device to a microscope!
3. **There's a clog somewhere**
    - **a. General tips:**
        - i. You should always make sure to filter your flush solution – small particles may accumulate and create difficult clogs
        - ii. It often helps to add a very small amount of surfactant, such as Triton-X 100, to the flush solution to help remove sticky debris.
        - iii. If worm density is too high at the entry into the device, the worms themselves may be the source of the clog. It often helps to load worms at lower pressures or gently shake the tube immediately prior to loading them, as they will otherwise settle.
        - iv. If your worms are too large for the imaging channel, you may have issues flushing them once imaged. You will either need to adjust the staging of your worms or the channel size of the device itself.
    - **b. If you can see the clog in the device:**
        - i. Turn on the flush. The added flow is designed to help flush out debris.
        - ii. If it's still there, gradually increase pressure through the main channel to dislodge the debris.

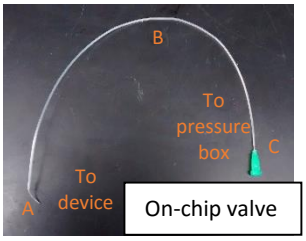
c. **If you can't see the clog in the device:**
    i. Sometimes clogs result when the bits of PDMS removed during the hole punching process are not completely removed, or incomplete punching of the PDMS at the glass-PDMS interface. Make sure during fabrication that the punched hole is cleared of obstructing PDMS and other particles.
    ii. Check your tubing, especially if you have used the same tubing before. If any liquid has been in the tubing before, evaporation can leave sediment in the tubing. If you must reuse tubing, push several milliliters of dH_20 or your favorite minimal buffer through it in advance of connecting it to the device to check for blockages.

You are ultimately responsible for your lab's microscopy equipment, so **practice** and **use your best judgement** for your particular situation. Happy sorting!

Tubing How-To for Sorting Device:

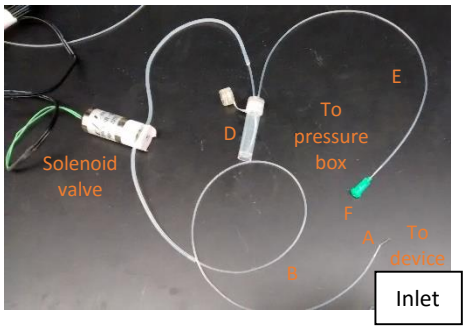
<u>Tubing set-up</u>

- On-chip valve tubing (#3, 4, 6, 8)
    A) Metal pin
    B) Polyethylene (PE) tubing
    C) Luer adapter



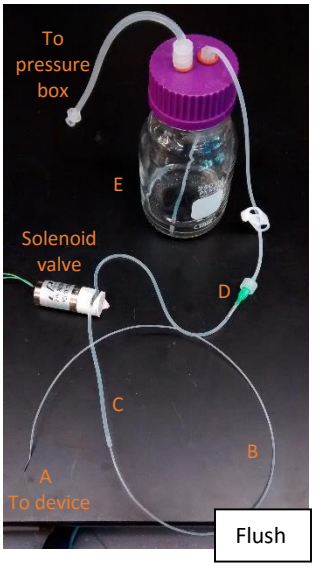
On-chip valve

- Inlet flow tubing (#2)
    A) Metal pin
    B) PE tubing
    C) Silicone tubing
        a. For use with the solenoid valve (part #29 on the pressure box parts list) for automation
    D) Loading inlet tube (see below to make loading tube)
    E) PE tubing
    F) Luer adapter



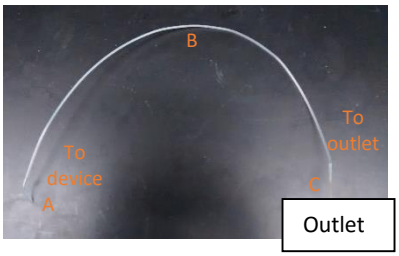
Inlet

- Flush channel tubing (#1)
  A) Metal pin
  B) PE tubing
  C) Silicone tubing
     a. For use with the solenoid valve (part #29 on the pressure box parts list) for automation
  D) Luer adapter
  E) Flush bottle (see below to make flush bottle)



Flush

- Outlet flow tubing (#5, 7)
  A) Metal pin
  B) PE tubing
  C) Metal pin



Outlet

<u>How to separate metal pins from the luer adapters</u>

Flame method

- Using tweezers to hold the adapter CAREFULLY heat the adapter over an open flame
- Quickly pull the adapter from the flame and use a second set of tweezer/pliers pull the metal pin from the slightly melted plastic component
- Melt off any remaining plastic from the metal pin
- If desired, gently bend the pin to ~30° to prevent tubing from entering your field of view

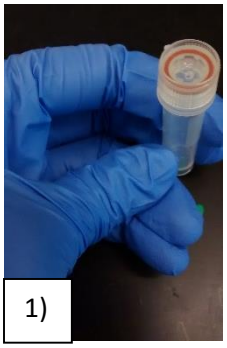
Plier method

- Using pliers, rotate the adapter and gently press down on the glue attaching the metal pin to the plastic adapter component
- Use pliers to pull the metal pin from the plastic adapter
- Use a razor to carefully shave off the remaining plastic from the metal pin
- If desired, gently bend the pin to ~30° to prevent tubing from entering your field of view

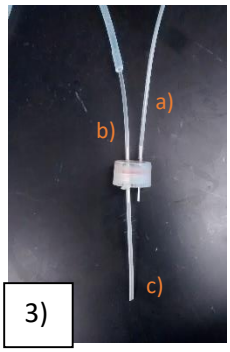
<u>How to make a loading inlet tube (for inlet channel)</u>

1. Make two holes in the lid of a 2ml microtube
   - Heat the metal adapter tip (21G) and push them through the lid to melt the holes

2. Place 2 21G metal pins midway through the holes and epoxy in place
3. After the epoxy is fully set, attach PE tubing to the pins.
    a) Tubing to the pressure box
    b) Tubing to the silicone tubing/device
    c) Tubing for worm loading (Note: make sure the tubing reaches the bottom of the microtube to ensure worm capture as shown in 4)


1)


2)

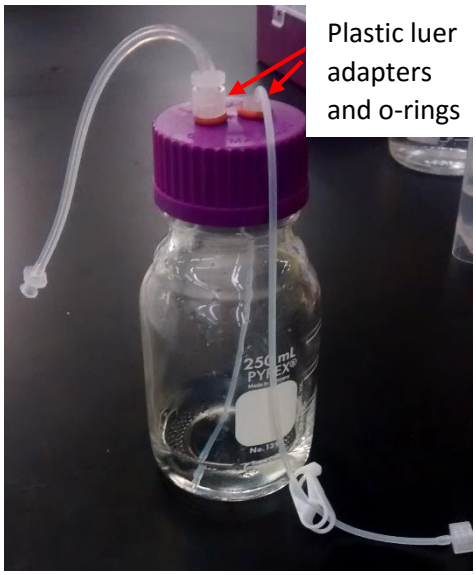


3)


4)

How to make a flush bottle (for flush channel)

1. Drill and tap 2 holes in the cap of a bottle
2. Screw 2 plastic luer adapters in the holes to connect the tubing
    i. Luer components ordered from Nordson Medical (http://www.nordsonmedical.com/products/luer_fittings.aspx)
    ii. Add rubber o-rings between the plastic luer adapters and the bottle cap to improve the seal between plastic cap and glass bottle
3. Attach silicone tubing to the luer adapters
    i. Tubing to the pressure box
        1. Luer adapter to connect from the pressure box to the tubing
        2. Luer adapter to connect from the tubing to plastic luer adapter screwed onto the bottle cap
    ii. Tubing to the flush tubing/device
        1. Luer adapter to connect from the tubing to remaining flush channel tubing
        2. Ensure that the tubing goes through the adapter and reaches the bottom of the bottle



Sorting Operating Procedure

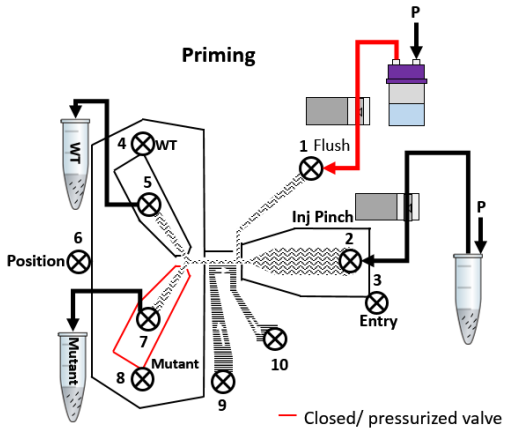
1. De-gas the on-chip valves

- Load the on-chip valve tubing (3, 4, 6, 8) with ~1 inch of the 50% glycerol, DI water solution
    - **NOTE**: Do NOT overload the line and let the solution get into the pressure box. It WILL damage the regulators within your box

- Plug in the tubing into the punched holes in the device (see diagram for tubing location), and connect the tubing to the pressure box. Ensure that the valve ID on the GUI matches the valve number on box
- Click “Valves” to initialize the communication between the valve box and GUI
- Pressurize the tubing (begin @ 15-20 psi and gradually increase until valves close)
- Click the corresponding valve button numbers (under Valves) to turn on the pressure to that line – check that de-gassing occurs

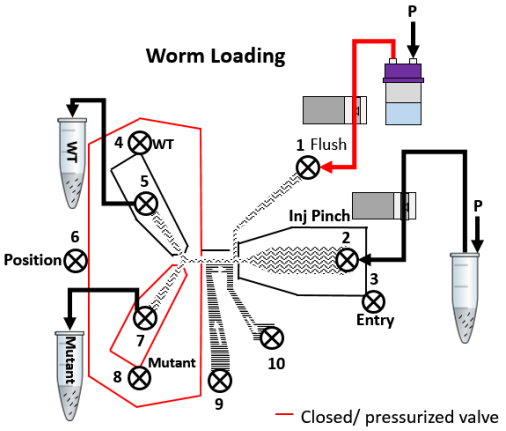
2. Connect the flow tubing into the corresponding holes in the device (see diagram for tubing location)

- 1) Flush channel tubing
- 2) Inlet channel tubing
- 5 & 7) Outlet PE tubing



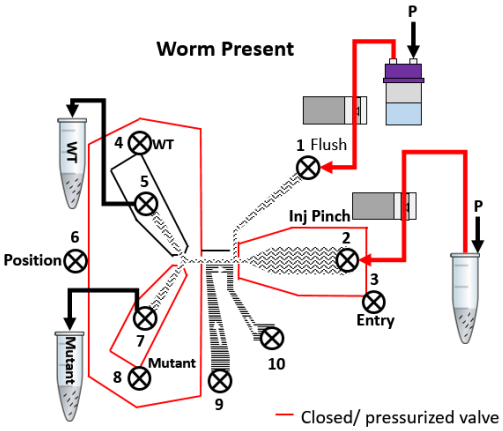
3. Prime the device (checking for debris/clogging in the flow tubing)

- Fill the loading tube w/ 0.5ml filtered M9 and connect to pressure box
- Set inlet pressure at 3-5psi on pressure box
- Press “Priming” on GUI to flow M9 through the device (see diagram on the left for priming chip operations)



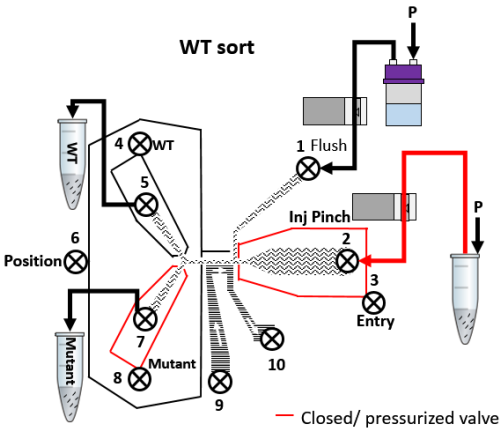
4. Worm Loading

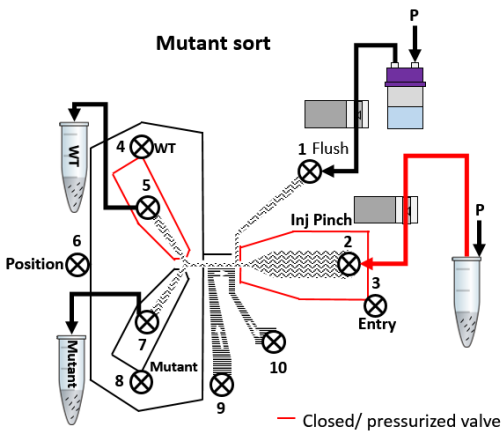
- Fill the loading tube w/ the population of interest
- Press “worm loading” on the GUI to begin flowing worms into the imaging channel (see diagram on the left for worm loading chip operations)



5. Worm Present & Sorting

- Press “Worm Present” on GUI when single worm is present in the imaging channel (see diagram on left for worm present chip operations)
- Decide if worm is WT or mutant (press either “Wild-type release” or “Mutant release” on GUI)
    - See diagrams for wild-type release or mutant release on chip operations below
    - Automated release times for valves can be changed in “release times” in GUI





Sorting GUI reference