



Stimulus Preference Chip

User Guide

[Apparatus and setup](#) (video available at nemamatrix.com)

1. Place the chip on the stage of a stereomicroscope, securing it with tape.
2. Connect fluid-reservoir syringes and vacuum sources to the chip as shown in Figures 1 and 2. A fluid head of approximately 30 cm is typical.
3. Fill reservoirs 1 and 3 with a stimulus-free buffer solution. This will be used to establish baseline behavior and flush the chip between experiments.
4. Fill reservoirs 2 and 4 with testing solutions. For example, reservoirs 2 and 4 might contain chemically distinct chemoattractants to compare their relative attractiveness.
5. Fill the worm loading syringe with about 5 mL of buffer. Ensure there is no air in the attached tubing, then insert the end into the appropriate worm port; this connection blocks the port to prevent leakage while the chip is being loaded with solutions.
6. Activate the outlet vacuum and run solutions 2 and 4 through the chip until there are no bubbles in the stopcocks, tubing, or chip.
7. Repeat with solutions 1 and 3.
8. Deactivate the outlet vacuum and close all stopcocks. The chip is now ready for loading a worm.

Worm Loading

1. Pick a worm to an unseeded agar plate.
2. Wash it with a drop of buffer, and allow it to crawl away from the drop.
3. Capture the worm in the end of the tubing of the loading syringe and insert it into the worm port.
4. Gently expel fluid until the worm is visible the near worm clamp.



5. Wait a moment until the worm's swimming motions cause its head to be pointed toward the clamp
6. Push the worm midway through the clamp.
7. Immediately activate the worm clamp vacuum. A well-positioned worm will exhibit sinusoidal movements of $1/4$ to $1/2$ wavelength at approximately the frequency of crawling on an agar surface.
8. Open stopcocks 1 and 3, and activate the outlet vacuum to run the buffer solution through chip. At this point you may wish to record baseline behavior.

Stimulation

1. To establish the stimulus condition,
 - Open the stopcocks on reservoirs 2 and 4,
 - Close the stopcocks on reservoirs 1 and 3 (buffer).
2. To terminate the stimulus,
 - Open the stopcocks on reservoirs 1 and 3,
 - Close the stopcocks on reservoirs 2 and 4 (buffer).

Removing the Worm

1. Deactivate the worm clamp vacuum.
2. Push the animal out of the clamp with the worm loading syringe.

Cleaning the Preference Chip System For Later Use

1. Disconnect reservoirs 1-4 from the chip, leaving the tubing attached to the syringes.
2. Fill each syringe with distilled water, insert the plunger, and push all the water out.
3. Repeat with isopropyl alcohol (IPA).
4. Remove the plunger and tubing and blow dry the syringe with compressed air.

5. Blow-dry the tubing by forcing compressed air through it. The chip is cleaned in similar fashion. Connect syringes filled with distilled water to the two fluid inlets.
6. Using the plunger, gently force several mL of water through the chip, first with one syringe then, the other. Fluid leaking out of unconnected ports can be absorbed with a Kimwipe.
7. Repeat with IPA.
8. To blow dry the chip, remove all tubing and connect a source of compressed air to the fluid outlet (~40 psi) for several seconds until visual inspection shows no IPA remains.
9. Seal the ports with Scotch Magic Tape to prevent collection of dust.

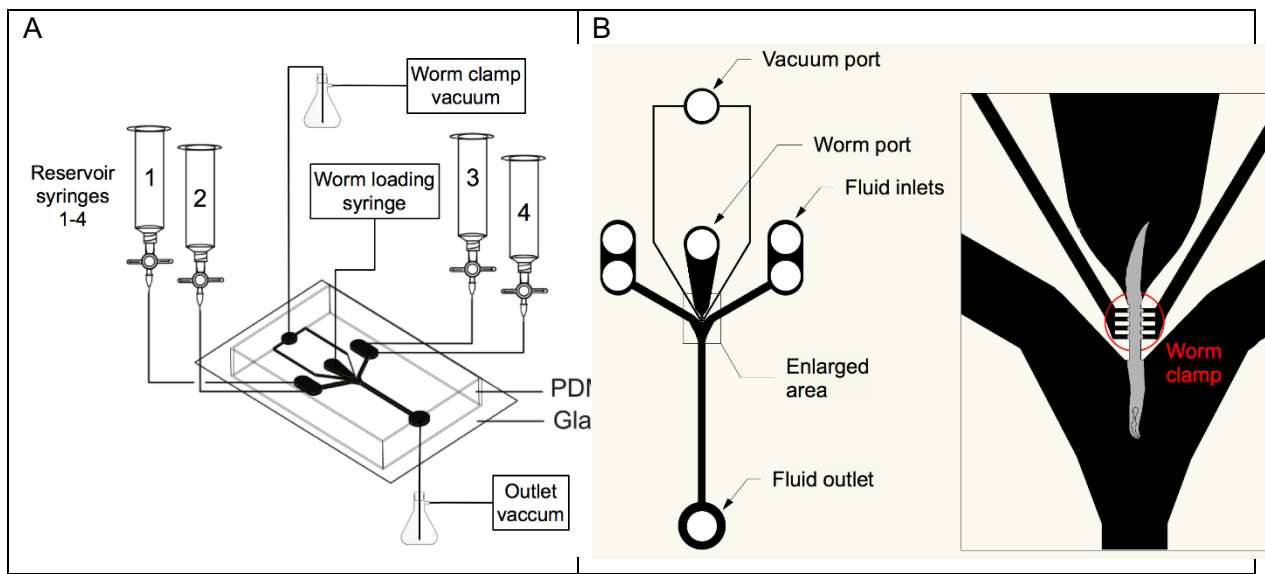


Figure 1. A. Overall view of the Preference Chip setup. For the worm clamp vacuum, use regulated house vacuum (~ 25 Kpa) or a diaphragm vacuum pump (e.g. DryFast, Model No 2014B-01, Welch Vacuum Technologies, Monroe, LA, USA). For the outlet vacuum, use regulated house vacuum, a second diaphragm pump, or a peristaltic pump (e.g., Model No. 426-2000, Labconco, Kansas City, MO, USA). Adjust the strength of the vacuum (or flow rate of the peristaltic pump), to achieve the desired rate of flow in the chip. If a peristaltic pump is used, the associated side-arm flask is omitted. **B.** Diagram of the chip's fluidic channels.

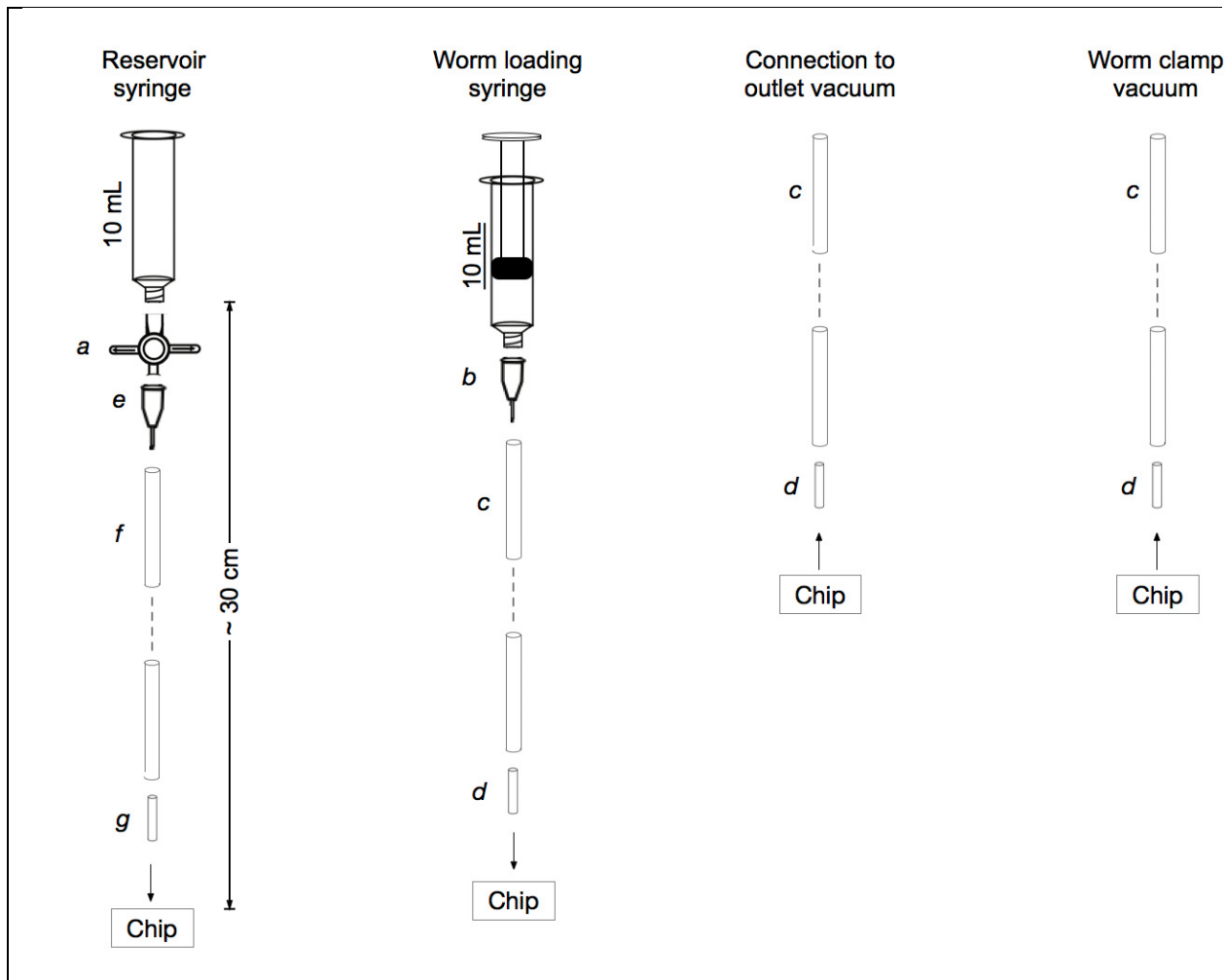


Figure 2. Fluidic connections to and from the Preference Chip.

a. 1-way stopcock (e.g., D100 455980, Braun Medical Inc., Bethlehem, PA, USA).

b. 17-gauge needle (e.g., P/N 75165A24 17Gauge 1/2"Lg. 0.048"ID 0.058"OD, McMaster-Carr, Santa Fe Springs, CA USA).

c. PE/9 polyethylene tubing (e.g., BB31695-PE/9, Scientific Commodities Inc., Lake Havasu City, AZ USA).

d. "1.5 mm" OD SS tube: P/N 17 TW x 0.500 in long (0.058 OD x 0.047 ID), Type 304 WD, Full Hard, New England Small Tube Corp, Litchfield, NH USA.

e. 20-gauge stub needle (e.g., P/N 75165A677 20Gauge 1/2"Lg. 0.026"ID 0.036"OD, McMaster-Carr, Santa Fe Springs, CA USA).

f. PE/6 polyethylene tubing (e.g., BB31695-PE/6, Scientific Commodities Inc., Lake Havasu City, AZ USA).

g. "1.0 mm" OD SS tube: 1.0 mm OD: P/N 19 TW x 0.500 in long (0.042 OD x 0.032 ID), Type 304 WD, Full Hard, New England Small Tube Corp, Litchfield, NH USA.