Isolating rotifers individually for DNA extraction

1) Label a clear Eppendorf or microcentrifuge tube with the date and a short individual identifier for each tube in the extraction set. Record which dish or sample the rotifers in each tube came from.

2) Using a 0.5-10µL pipette, transfer 8µL of sterile molecular water into the bottom of each isolation tube in advance. Avoid air bubbles; centrifuge the tubes briefly if necessary.

3) Move rotifers from the source dish or wash droplet using a **MOUNTED NEEDLE**. Hooked needles work best. Locate a single animal of interest under the dissecting microscope, flick it gently to detach it from the substrate and pull it to the water surface. Be careful not to lacerate or crush it!

4) At the water surface, draw the animal out of the water using the needle. **QUICKLY** move the needle to an isolation tube. Do not spend time putting the tube under the microscope; you do not need to see the transfer.

5) Dip the needle in and out of the water droplet gently **FIVE** times. Do **NOT** touch the side of the tube with the needle! Do **NOT** scrape the needle on the bottom of the tube; this could damage the animal.

6) **IMMEDIATELY,** bring the needle back under the microscope. Do **NOT** touch the side of the tube with the needle! While watching closely, dip the needle into the water on the source dish. If the animal was successfully transferred to the tube, it will no longer be on the needle. Turn the needle 360^{o} and look closely for any bump or translucent spot that might be the rotifer.

7) If you still see the rotifer on the needle, repeat Steps (4)-(6) until you no longer see it on the needle. Once you can no longer see the animal on the needle, you must assume it is in the tube. **SEAL** the tube for extraction.

8) There is little point checking the isolation tube under the microscope; rotifers are tiny, transparent and often contract after being moved. They cannot reliably be detected in a water droplet in a tube. Even if you cannot see it in a tube, you **MUST** assume it went in and seal the tube for extraction. **NEVER** attempt to transfer a second animal to the same tube, even if you think the first did not go in! If there is any uncertainty, discard the tube and start a new one.

9) Sterilize the needle between **EVERY** transfer. Hold it briefly in the base of a lighter flame until it glows red.