**Planarian Worm Water Protocol**

**1.** Autoclave an empty container, with a fresh piece of autoclave tape. 15 L polypropylene carboys with a spigot work well for this. Make sure that the lids/caps are loosely threaded (not tightly closed) while autoclaving, or the carboys will become misshapened. Do not autoclave the spigot itself (instead use the alternate cap provided).

 --Caution: do not use soap on anything that will come into contact with worms.

 --Autoclave the container between each batch of worm water.

 --Autoclave the container “dry” (without water).

 --Autoclaved containers are good to use if black stripes appear on the autoclave tape.

**2.** Fill the autoclaved (and cooled) carboy with 15 L of type 1 ultrapure (18.2 MΩ-cm) water.

 --There are many purification systems for this (depending on the system, they start with either deionized or tap water and include UV treatment and a final 0.2 μm filter).

 --Alternatively, filter deionized water through a 0.2 μm membrane filter.

**3.** Add 0.5 g/L (or 7.5 g per 15 L) of ocean sea salts (see table of materials).

**4.** Close carboy lids/caps and shake several times to mix.

**5.** Date the carboy (on the piece of autoclave tape). Typically use carboys within 2 weeks. Make sure that carboys are tightly sealed when not in use, to prevent evaporation (which changes the salt concentration).

**6.** When the carboy is empty, fill 1/8 full with ultrapure water, closed lids and shake to remove any accumulated salts from the insides of the carboy. Drain completely. Remove old, dated autoclave tape. Carboy is now ready to be re-autoclaved for next use (see Step 1).

 --If not autoclaving immediately, store with lids/caps loosely threaded to allow carboy to dry completely.

**Variations**

**a.** If an ultrapure water source is not available, worms can be kept in bottled spring water. Most brands of bottled water are adequate for short term worm housing (<3-4 weeks). For longer housing, you may have to test several spring water brands to find one the worms prefer. Poor water (*i.e.* with unfavorable salt concentrations) will result in worms that form lesions, undergo head regression (where just the head lyses), or die. Optimal water will result in little to no death or lesions, and a healthy mix of whole and regenerating worms (*i.e*. asexually reproducing worms that have fissioned into a head and a tail fragment).

**b.** The use of a low-profile carboy (which is wider than it is tall) makes filling worm colony boxes much easier. Place the carboy on a small shelf, so that the carboy is raised above the plastic food container (which sits on the counter underneath).

**c.** If a carboy is not available, worm water can be made and stored in glass containers. Just make sure that the glass containers are tightly sealed to avoid evaporation, and that they are never washed with soap (which is lethal to worms).