# Planarian Care and Maintenance

Pearson Lab, March 15, 2011

(modified from Sanchez and Newmark lab protocols)

"The clonal lines of Schmidtea mediterranea have now been maintained in the laboratory for over ten years using the methods outlined below; if you follow these instructions carefully, your planarians should grow and thrive. Remember, these are your pets and they require time, patience, and some TLC (fortunately, they don't need to be walked and they don't shed). Without healthy planarians, we will all be out of business." (Phil Newmark)

# Water

Bret Pearson and Kyle Gurley developed an Instant Ocean (IO) based Milli-Q planarian water around 09/2010. We recommend new colonies **(asexuals and sexuals**) be established in this water.

Here are the final concentrations (or mass, in the case of IO) of salts used:

$MgSO_4$ 0.83 mM
$CaCl_2$ 0.9 mM
$KHCO_3$ 0.04 mM
$NaHCO_3$ 0.9 mM
Instant Ocean salts: 0.21 g/L

The following stock solutions are prepared with MilliQ water and filtered through a 0.22 µm filter top:

1 M $MgSO_4$
1 M $CaCl_2$
0.1 M $KHCO_3$
0.5 M $NaHCO_3$
21 g/L Instant Ocean salts

You can use the same filter top for all solutions. For a 15 L carboy, add the following volumes of each stock solution:

12.5 mL $MgSO_4$
13.5 mL $CaCl_2$
6 mL $KHCO_3$
27 mL $NaHCO_3$
150 mL Instant Ocean salts

You could probably get away with preparing a 1 M stock of $NaHCO_3$, but we don't do it. Start filling the carboy with water before adding in the salts. We autoclave empty

carboys after two refills, as a precaution against growth of nasties. We do not autoclave full carboys.

# Containers

Planarians are maintained at 18-22°C in the dark in Ziploc containers (for 2L containers, MAX 5000 worms in 1500ml). The lids should not be sealed, but rather should be left ajar or loose (for airflow and mold control). The containers should not be treated with any detergents or other chemicals that can leave a residue. When starting a new container of worms, make sure to first wash the container out with 70% ethanol and let dry. Planarians are extremely sensitive to environmental contamination; the cleaning and feeding procedure detailed below will suffice to keep the containers clean and the planarians happy.

# Liver Prep

Obtain an intact organic calf liver. Preferably not older than 48hrs, but the fresher the better. Take liver out of packaging and rinse off extra blood with MilliQ or RO-H2O water. The subsequent steps are done on cleaned cafeteria trays. Try to keep liver on ice as much as possible.

Remove the outer capsule. Good livers should have strong capsules that peel off easily in large sheets.

Liver can be cut into large pieces and given to multiple people for further processing.

"Large" veins need to be removed. Everyone has their own method but some popular ones are: 1) Take a normal razor blade and scrape the liver off of the veins. 2) Use a scalpel to cut liver off around the veins. 3) Use pure hand power and squeeze liver off of the veins like pulling leaves off of a stem. An important point here is the "principle of diminishing returns". You could go crazy and spend forever getting out all the veins, but it doesn't really matter at some point. 3 people working on this should only take about an hour (even for novices). All liver that is separated from connective tissue should go into beakers on ice.

Blend/food process liver chunks. Only blend to the point where the chunks are broken up, but not to the point where you are getting a smoothie-like texture (and probably too much heat as well).

Liver should now be worked through some sort of fine metal mesh to remove the remaining small pieces of connective tissue. There are several ways to do this. In the Sánchez lab, we had syringe-attached filter housings (can buy the housings through VWR). The liver was put into 30ml syringes and passed through a course metal mesh, then a fine metal mesh. I found this process difficult and messy and exhausting. Since then, we have switched to fine metal colanders and wooden spoons. While still exhausting, this process is much faster, has better liver recovery, and has much less plastic waste. You want to pick up your colanders at a good kitchen store and buy a diameter that fits the top of a 4L Nalgene plastic beaker. Work the liver through the colander over the plastic beaker on ice until the remaining liver looks less like liver and more like liver-colored connective tissue. Periodically, use a new spoon to scrape off the filtered liver on the bottom of the colander. Throw away connective tissue.

**ALTERNATIVE NEWMARK LAB METHOD: Buy a hand-cranked food mill. Put whole chunks of liver into the food mill, capsule, veins, and all, and just crank it through, washing periodically. This is so fast and easy it can be done in the cold room. We do this now and have had no problems. Way to go Phil's lab on this one.**

Load liver into 250ml Nalgene bottles that can fit into a swing bucket rotor. Spin for ~5min at MAX speed. This is to remove air bubbles, which if present, will make the food float in water = SUPER annoying. Note: fixed angle rotors will send liver flying everywhere if bottles are overly full or have liver residue on the lids/sides.

With a pipetaid and 25ml pipet with tip removed, aliquot liver into small petri dishes (35mm). About 5ml per aliquot.

Stack petri dishes in freezer boxes. When full, it is ideal to vacuum seal the boxes. Not necessary though. Unsealed boxes are good at -80°C for ~6 months depending on where you live. Utah had a shelf life of about 6 months. Toronto is > 1 year. Vacuum sealed last slightly longer. We don't know why, but if the food "goes bad", worms will simply stop eating it.

# Worm Feeding and Maintenance

For projects in the lab that aren't based on screening large numbers of genes, approximately 5 well-stocked boxes of worms will suffice per person. Both in situs and RNAi experiments use worms that are starved >4 days, so every week that you will do experiments (every week!) you should keep at least 1 box starved. Rotate which box is starved every week.

For population maintenance, feeding once per week is sufficient. For population expansion, feeding twice a week is necessary.

When expanding the population, feed twice a week until worms are big, then amputate cleanly into 3rds (this will minimize regeneration time and head/tail fragments will eat in about 10 days, and trunks will eat in about 5 days). Repeat until you have enough worms.

**For feeding:**

Thaw a petri of liver paste. 1 petri of food will easily feed 5 boxes of worms. Before the liver is completely thawed a metal spatula can be used to break off chunks of liver. 2 chunks per box at opposite ends is enough. Unused liver can be put at 4°C and used for up to 2 days.

Worms will be done feeding in ~1hour. Once worms are finished, remove remaining liver. Modulate how much you give your boxes based on how much liver is remaining. If there is a lot remaining, give less next time.

**For Cleaning**

There are 2 types box cleaning: 1) water rinse 2) wipedown.

**Rinse:** Pour off all old water. Rinse with ~200ml of new water. Refill box.

**Wipedown:** Pour off all old water and get worms into the corner. Wipe all mucus off of the container with a paper towel. Rinse worms with ~100ml of water into opposite corner of box and pour off water. Wipe the remaining part of the box free of mucus. Refill box.

When worms are fed, they undergo a wipedown that day and are rinsed 2 days later. Starved worms get a wipedown once per week.

**Strategies to maintain population size:**

1) When removing X number of worms for an experiment, cut X number of remaining worms so the net change is 0.
2) Perform regular (~monthly) mass-cuttings to keep pace with experiments.

**Exceptions to the above cleaning schedule:**

1) High-density cultures. These can produce significantly more mucus and may need an additional wipedown per week. Applies to boxes with >10K worms. High density does not appear to affect worm health if boxes are kept clean.
2) After mass-amputations. If you have several thousand worms in a box and you cut each into multiple fragments, a LOT of mucus will be made over the next couple days. It is a good idea to do a wipedown 2-3 days after a mass-amputation.
3) Most important tip: it is possible to over-clean and over-handle the worms. Refrain yourself from adding additional rinses and wipedowns and feeds to the above schedule as you will likely only make things worse.
4) Occasionally you will have bacterial or protozoan blooms in your boxes that make your worms sick (i.e. stop eating and/or lose their heads). As soon as you see this, it is important to do a wipedown, and the wipedown can be done with a papertowel sprayed with some 70% EtOH. Get worms on Gentamycin (50 µg/mL diluted in their normal water from a 50 mg/ml stock) immediately and keep them in it for 2 weeks. Add another wipedown a few days later and monitor the situation closely. When headless worms are on the recovery they will produce blastemas immediately and should be up and feeding in about a week. If health issues were caused by water problems, try a different source of MilliQ water immediately and get worms into new water ASAP. Perform additional wipedowns if boxes become excessively dirty or have planarians dying.

**Troubleshooting (mainly from older worm-care protocols):**

If the animals are secreting excessive mucous, they are probably not happy with their situation. You can tell that the mucous is overabundant if it is difficult to remove intact animals from the surface of the container by gently squirting them with water from a disposable pipette. Likewise, they will adhere too firmly to the surface of the Petri dish when you are cleaning their container.

If you see black spots on the planarians, this is also a bad sign. If the animals develop lesions or actual holes through the animal, cut the worm anterior and posterior to the lesion and let the cut pieces regenerate (discard the lesion area),

Even worse is when the flatworms begin to lyse in the head region. You can distinguish this from animals that have recently fissioned and are regenerating due to the fact that the fission fragments generally come from the area behind the pharynx, whereas the lysing planarians will look like they are regenerating a head in front of the pharynx. This disintegration is usually accompanied by an awful smell.

In all of these cases, the sick animals should be separated from the healthy animals and transferred to a clean container with planarian water supplemented with

gentamicin (stock at 50mg/mL and use at 1:1000 in final worm water). This helps prevent opportunistic bacterial infections that can contribute to the demise of less-than-100% healthy planarians. Change their water and clean their container often, being sure to keep all pipettes separate from those used with the rest of your cultures.

If the animals dislike the water they will crawl out and mass on the side of the container, presumably as a protection against dehydration. Change the water whenever this behavior is observed. This should not be confused with the photophobic behavior of planarians, which will cause them to bunch behind anything that offers shade (e.g. labels and decorations on the sides of the containers).