How to Make an Inexpensive, Low-Temperature Incubator Using a Refrigerator and Temperature Controller

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Preface

In this manual, we describe how to make a relatively inexpensive ($10 to $100) low-temperature (around 15°C) incubator for storing live cell cultures over prolonged periods of time (e.g., for semester- or year-long research projects or lab activities). Specifically, we use a standard dorm room refrigerator or “mini fridge” plugged into a commercial temperature controller, which can maintain a relatively stable internal environment. In addition, we include a medium-sized (e.g., 250- or 500-mL) glass beaker filled with water and containing a thermometer inside the refrigerator to help maintain and monitor the internal temperature. As with other open source instruments and pieces equipment that we have developed, http://pages.stolaf.edu/opn-lab/equipment/, we call this design the low-temperature version of the “OPN Incubator,” and we hope that it will help schools with limited budgets expand the type of scientific tools available for their students (and, thus, the types of experiments and studies that these students can conduct during the school year). We further encourage others to modify or improve upon this design to fit their particular educational or research needs.

Introduction

The ciliated protozoan *Tetrahymena thermophila* is a common single-celled organism that is often found in fresh-water lakes and ponds (Carter and Cameron, 1973; Lang and Kohidai, 2012). Given their cellular anatomy and physiology, as well as the ease with which these cells can be cultured and stored, *Tetrahymena* serve an ideal model organism for teaching students a variety of different biological concepts as well as basic scientific and research skills (Bozzone, 2000; Smith, Wiley, and Cassidy-Hanley, 2012; see also our laboratory manuals describing different educational experiments that can be conducted with *Tetrahymena* at http://pages.stolaf.edu/opn-lab/experiments/). However, without a temperature-controlled environment, storing live *Tetrahymena* cultures for prolonged periods of time (such as for semester- or year-long research projects) can prove rather difficult.

Indeed, like other unicellular organisms, live *Tetrahymena* cells have a fairly narrow range of optimal storage temperatures, which are below normal room temperature (roughly 23°C). Specifically, a cooler temperature slows down the growth rate of these cells, enabling a given culture to last much longer than it otherwise would at a higher temperature. In our lab, we have found that live *Tetrahymena* cells can be stored for many months at 15°C. However, if the temperature drops much further below 15°C for prolonged periods of time (e.g., to around 5°C), then the cells can easily die. As a result, when storing live *Tetrahymena* cultures for educational or experimental purposes, it is very important to keep the cells at a relatively stable temperature, which is neither too hot nor too cold.
Although a number of scientific and industrial companies make temperature-controlled incubators that can fit these needs, these models often cost in the hundreds (or thousands) of dollars. Not surprisingly, these expenses can place this equipment out of reach for many schools (particularly those with limited financial resources), which constrains the types of educational experiences that these schools can provide to their students. Similarly, while a variety of alternative incubator designs for a wide range of applications have been discussed in the literature, these models are often too large (Martinez and Agosta, 2016), too small (Heidemann et al., 2003; Picard et al., 2010; Tazawa et al., 2016), or too complex (Elliott and Heiniger, 1965; Clark, Kaminski, and Karzon, 1970) to fit the particular storage needs described here.

As a result, we have prepared this short manual to explain how readers can build their own low-temperature incubator at a fairly low cost ($10 to $100) and then use this incubator to store live cell cultures for classroom demonstrations, teaching exercises, or other research projects. Specifically, we have found that, by plugging a common dorm refrigerator into a standard temperature controller, readers can maintain multiple Tetrahymena cultures at a fairly stable temperature (around for 15° C) for months on end. Indeed, we have used this set-up in our lab for nearly one year now (as of October 2016), and we have not run into any issues with the cell lines that we have used.

Depending upon the exact make and model of the equipment used, including whether it is new or used, the entire set-up should cost between roughly $10 and $100 in total (not including any taxes or shipping costs). Like other instruments and equipment that we have developed, http://pages.stolaf.edu/opn-lab/equipment/, the plans and parts for this design are all open source, so we have named the model the low-temperature version of the “OPN Incubator.” Ultimately, we hope that this design will be helpful to teachers or researchers at many different levels and schools, and we invite others to use or expand upon these basic plans to suit their particular educational or research needs.

Materials

To make our low-temperature version of the OPN Incubator, we used a Criterion® 1.5 cubic foot compact refrigerator with an internal freezer that we purchased at a regional home improvement store for roughly $75. However, readers could use other “mini fridges” (or even larger refrigerators) instead, including used models that are frequently available for free or at very low prices (e.g., $1 to $50) on websites like https://www.craigslist.org or www.eBay.com. Readers can also look for used refrigerators at garage sales, in the classified ads of local...
newspapers, or by posting “wanted signs” on the campuses of local colleges or universities near the end of the school year.

For our temperature controller, we used a new WillHi Model No. WH 1436, which we purchased on eBay for approximately $25. Again, however, readers could use other models or brands instead, and we have found that digital temperature controllers typically cost between $8 and $35 each online (at September 2016 prices).

Also, for those unfamiliar with temperature controllers, these devices work in much the same way that a thermostat does. In short, by plugging a refrigerator into a temperature controller and the controller into a wall socket, the controller can then turn the refrigerator on (or off) when the appliance becomes too warm (or too cold) inside. Specifically, because the controller has an external sensor (called a “thermocouple”) that can be placed inside the refrigerator, the device can both measure the internal temperature of the appliance and then take steps to adjust that temperature (to a pre-set level) as needed. As a result, the refrigerator can stay at a relatively stable temperature (one that is pre-programmed into the controller) over time.

**Directions**

To set up the low-temperature version of the OPN Incubator, first plug the refrigerator into the temperature controller and the controller into the wall socket (Fig. 1). Also, we set the thermostat dial (located on the back of our Criterion refrigerator) to “3” (on a scale of 0 to 5) to provide a moderate environment inside the appliance. Readers, however, may need to choose a different value based on the scale listed on their dials or their specific storage needs.

Next, follow the written instructions that accompany the temperature controller to set the desired temperature level (as well as any higher or lower “bounds,” any “alarm” settings, and any minimum or maximum settings for the device). Readers may also want to test their controllers at this point to make sure that they provide the appropriate warnings or alerts when the recorded temperature rises above or falls below the pre-programmed limits.
Figure 1. Plugging the refrigerator into the temperature controller (here, a WillHi Model No. WH 1436).

After setting up the refrigerator and temperature controller, attach the thermocouple (the white cord on the right side of Figure 1) to the inside of the refrigerator with tape (Fig. 2). For simplicity, we run the thermocouple along the side and top of the refrigerator (away from the back) and then across the door frame, so that the door closes over the cord (Cover Image; Figs. 2 and 3). Also, we do not recommend draping the cord for the thermocouple over the back of the refrigerator in case the electrical and mechanical equipment located back there might interfere with the sensor.
Figure 2. The thermocouple taped to the inside of the refrigerator.

We further use standard tape for labeling glassware to hold the thermocouple in place (both inside and outside of the refrigerator) since this kind of tape can be easily removed in the event that the thermocouple needs to be repositioned (Fig. 2). Of course, readers can use other types of tape instead, including heavy duty tape (e.g., duct tape), which can provide a more secure or permanent connection.

We typically place the thermocouple near one side of the refrigerator (on the opposite side of the ice box) slightly above the middle of the chamber, but away from the wall (Fig. 2). We use this location, so that the thermocouple will be near the center of the refrigerator while still having enough empty space around it to allow for undisturbed temperature readings. That way, the sensor is unlikely to come into contact with the inside wall or any media tubes, metal caps, or test tube racks – all of which could lead to spurious internal temperature readings that might throw off the controller.

In addition, before storing any cell cultures in the OPN Incubator, we place a medium-sized (e.g., 250- to 500-mL) beaker filled with water and containing a thermometer in the refrigerator to help stabilize and monitor the internal temperature (Fig. 3). We then regularly
check on this temperature over the next several days, while adjusting the levels on the controller as needed, to make sure that the internal environment has equilibrated around its desired level (15°C) before we start placing cell cultures in the incubator.

![Image of OPN Incubator]

**Figure 3.** The contents of our OPN Incubator. Tubes containing stock Tetrahymena cells in media (left) and a 200-mL beaker of water with a thermometer (right).

In our view, this approach is preferable to simply placing a thermometer inside of the refrigerator because the air temperature inside the appliance can change quite rapidly once the door is opened. Instead, if placed in a container full of water, the thermometer should display the temperature of liquids inside the refrigerator, providing a better indication of the environment surrounding the live cell cultures. Readers can also try placing the thermometer in a media tube full of water to better simulate the growth conditions of the cells. Nevertheless, we do not recommend using an open tube full of media because, even if previously autoclaved, the nutrient-rich environment of the media could provide a breeding ground for bacteria or other parasites, which could infect the surrounding cell cultures.
Discussion

As explained above, we set our OPN Incubator to maintain a 15° C (i.e., 59° F) environment for storing our *Tetrahymena* cultures. Again, however, before placing any live cells in the incubator, we spent a few days monitoring the internal temperature of the refrigerator and adjusting the temperature ranges on the controller to “dial in” the 15° C value. In our view, this step is necessary to ensure a well-calibrated internal environment, and readers should not simply rely on the values displayed on their controllers since a variety of factors can lead to a difference between the ambient air temperature inside the refrigerator and the actual temperature of the cell cultures stored in the appliance.

Indeed, depending upon the exact equipment used, readers may find it necessary to set their “target” temperature and related “bounds” on their controllers slightly above or below the desired value to achieve the ideal result. For example, as shown in Figure 1, we had to set our temperature controller slightly above 15° C (59° F) to maintain that temperature internally. Nevertheless, we have found that, once calibrated, our OPN Incubator can hold its temperature for prolonged periods of time. Specifically, our set-up has been running for nearly one year (as of October 2016), and we have yet to experience any issues with our cells or the internal environment.

In addition, while we set our temperature controller to maintain a 15° C internal environment for our *Tetrahymena* cultures, these controllers are often able to operate over a wide range of temperatures (e.g., from at least -20° C to 105° C for the WillHi WH 1436). Of course, many commercial refrigerators likely will not function over such a broad range, so readers should make sure to choose an appliance that will fit the specific storage needs of their model organism at the outset. In the process, readers should further keep in mind that, for this particular (low-temperature) application, the maximum internal temperature of the OPN Incubator will likely be capped by the surrounding room temperature.

Also, while we purchased a new refrigerator for our OPN Incubator, readers can instead purchase less expensive used ones online or elsewhere for very reasonable prices (e.g., from $1 to $50), and some used models may even be available for free. Similarly, the $25 digital temperature controller that we purchased online can be replaced with any appropriate controller, and there are less expensive new digital models available on eBay, Amazon, or other websites that range in price from roughly $8 to $20. Alternatively, readers can use older analog controllers, which can at times cost less than newer digital ones.

Relatedly, although we use a small “dorm fridge” for our OPN Incubator (Fig. 3), readers could use larger refrigerators instead. If so doing, however, we recommend placing multiple
containers of water in the appliance (with accompanying thermometers) given the larger internal environment. Readers should also consider refilling their containers with fresh water on a regular basis to avoid the growth of bacteria or other contaminants in the refrigerator. For similar reasons, readers should occasionally clean and disinfect their incubators, making sure to remove the cultures beforehand.

On a related note, readers should know that they will not be able to store their live *Tetrahymena* cultures in this set-up indefinitely. Instead, readers should use their old cultures to “seed” new ones every 3 to 4 months. To do so, first confirm that there are in fact live cells in the “old” culture tube (e.g., by holding the tube up to the light, it is usually possible to see the cells swimming below the surface of the media). Any cells located at the bottom of the culture tube after several months, however, are likely dead, so please do not take a sample from there. After confirming that there are live cells in the “old” tube, take a 500-µL sample of these cells from just below the surface of the media, transfer the cells to a “new” tube containing fresh media, and repeat this process for each of the old tubes. Then, as a safety precaution, put the old tubes back into the incubator and store them there overnight. Meanwhile, let the new tubes sit out in a rack on a table top at room temperature for the next 24 hours, so that the new culture can begin growing. After confirming that the new cultures have grown substantially, place the new tubes into the incubator and clean out the old ones for later use. If the cells have not noticeably multiplied overnight, leave the new tubes out on the table top for another 24 hours and check them again, or try seeding another set of new cultures from the old tubes that are still in the incubator.

Finally, while some readers may want to drill a hole into the top or side of their refrigerators and then feed the thermocouple through there (instead of using tape to secure the sensor in position), we do not recommend this approach for several reasons. First, without knowing the exact location of the internal components of the appliance (including any electrical wires or tubes containing Freon), drilling such a hole could be extremely dangerous. Second, making this type of modification to a new refrigerator would likely void its warranty. As a result, the appliance could not be returned or exchanged if it failed for some other reason during the warranty period (assuming, of course, that it would be appropriate to return or exchange the appliance given its prior contents). Third, such a hole should further be insulated and sealed to preserve the integrity of the internal environment. However, any permanent, airtight seal would likely prevent the thermocouple from being easily replaced in the event that it failed at some point in the future. Finally, as a practical matter, we have found that drilling this type of hole simply is not necessary to maintain a stable temperature inside the refrigerator. As a result, to us, this step would seem to provide little additional benefit given all of the associated costs.
Hazards

If using tape to secure the thermocouple inside the refrigerator, then there should be no significant hazards associated with making this low-temperature incubator. Nevertheless, readers should still make sure that they are using refrigerators and temperature controllers that are rated for their outlets (e.g., 120 Volts and 60 Hz in the United States). Also, as previously explained, depending upon what cells or organisms have been stored in the incubator, it may not be possible to return or exchange a new refrigerator even if it fails during the warranty period (due to the potential biological hazards involved). As a result, before attempting a return or exchange, readers should consult their chemical hygiene officer or other appropriate source. In addition, given the significant risks and other reasons described above, we do not recommend drilling a hole through the refrigerator cabinet and then threading the thermocouple through there.

Disclosures

The authors declare that they have no conflicts of interest related to any product, brand, company, website, or other item discussed in this manual. In fact, as with other open source instruments and equipment that we have developed, http://pages.stolaf.edu/opn-lab/equipment/, we encourage readers to experiment with different items to improve upon this design.

References


