Tested Studies for Laboratory Teaching
Proceedings of the Association for Biology Laboratory Education
Vol. 32, 237–244, 2011

This workshop was presented at the 31st ABLE meeting.

Teaching with *Chlamydomonas*

Mike Adams

Biology Department, Eastern Connecticut State University, Willimantic CT 06226 USA
(**adams@easternct.edu**)

The unicellular alga *Chlamydomonas* offers many advantages in undergraduate laboratory exercises. It is cheap, easy to maintain, non-hazardous and can be used to demonstrate a wide range of biological principles. Experiments involving regrowth of flagella and the sequence of events in the mating process are described, and a variety of variations on these exercises are suggested. Links to both research and teaching sites offer information on a wide range of additional experiments and suggestions for student independent study projects.

Keywords: *Chlamydomonas*, genetics, cell biology, student projects

Introduction

Adding new exercises to courses tends to be limited by several factors. The exercises should be relatively cheap, give reproducible and reliable results, be fairly simple from a preparation standpoint, offer the students an interesting and biologically meaningful experience and be expandable to add depth to the exercise if needed. All of these requirements are met in the exercises using *Chlamydomonas* that are described here and on the **Chlamydomonas Teaching Site**:

http://nutmeg.easternct.edu/~adams/ChlamyTeach/

Chlamydomonas is a single-celled, biflagellate green alga, approximately 10 µm long. The genus *Chlamydomonas* contains several species that have become popular as research tools, but by far the most frequently used is *Chlamydomonas reinhardtii*. As both a research and teaching organism it offers several advantages:

- It is nutritionally simple and cheap to grow, and can be grown photosynthetically, heterotrophically or mixotrophically.
- It is eukaryotic, but can be handled by standard microbial techniques
- It is motile and shows simple behavioral traits
- It can be grown as synchronous or asynchronous cultures
- It has a fast mitotic life cycle and also undergoes controlled sexual reproduction
- It has excellent genetics. Nuclear, chloroplast and mitochondrial genomes all have available markers
- All three genomes can be transformed; the nuclear genes can be transformed by very simple technology
- A wide range of mutant stocks and DNA clones are available from the *Chlamydomonas* collection
- Long term storage in liquid nitrogen is possible
- It is supported by the Genetics Society of America
- It is pretty, non-pathogenic, doesn't stink, doesn't contaminate other cultures and no one cares if you kill it

The basics for growing and maintaining stocks of *Chlamydomonas* are described and two fundamental aspects of *Chlamydomonas* biology; mating and flagella function, are the basis for some simple experiments, as well as the starting point for more sophisticated experiments and long term projects.

Student Outline

Mating and motility in *Chlamydomonas*

Background information

In this exercise you will look at two aspects of the biology of the single-celled haploid green alga *Chlamydomonas* (Fig. 1).

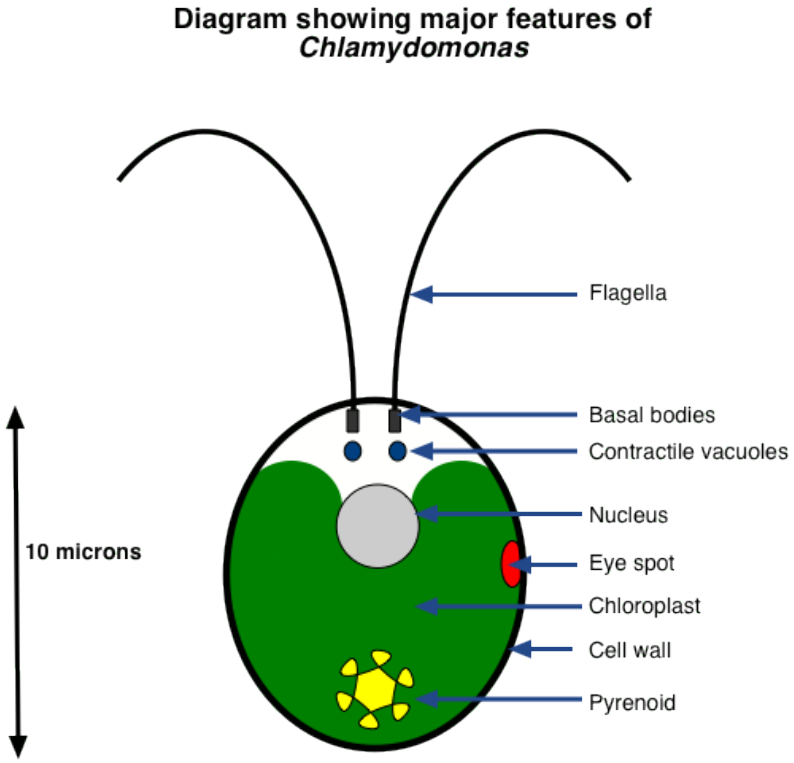

Figure 1. Diagram of the major cell structures visible in a light microscope.

Today's exercise will concentrate on two functions of the flagella, as described later. The basal bodies are needed for flagella growth. Contractile vacuoles help the cell to maintain proper osmotic conditions by pumping out excess water. The eyespot is light-sensitive and aids the cell in phototaxis (though it is not the only factor and cells lacking eyespots can still orient to the light). The pyrenoid is a starch-storage area in the chloroplast. The nucleus, chloroplast and cell wall all play roles similar to any other plant cell.

Reproduction

Although *Chlamydomonas* is a single-celled organism, it has two sexes (usually referred to as mating types and called plus and minus). As long as *Chlamydomonas* is well nourished the cells will grow and divide by mitosis (Fig. 2), so that all the descendants are genetically identical.

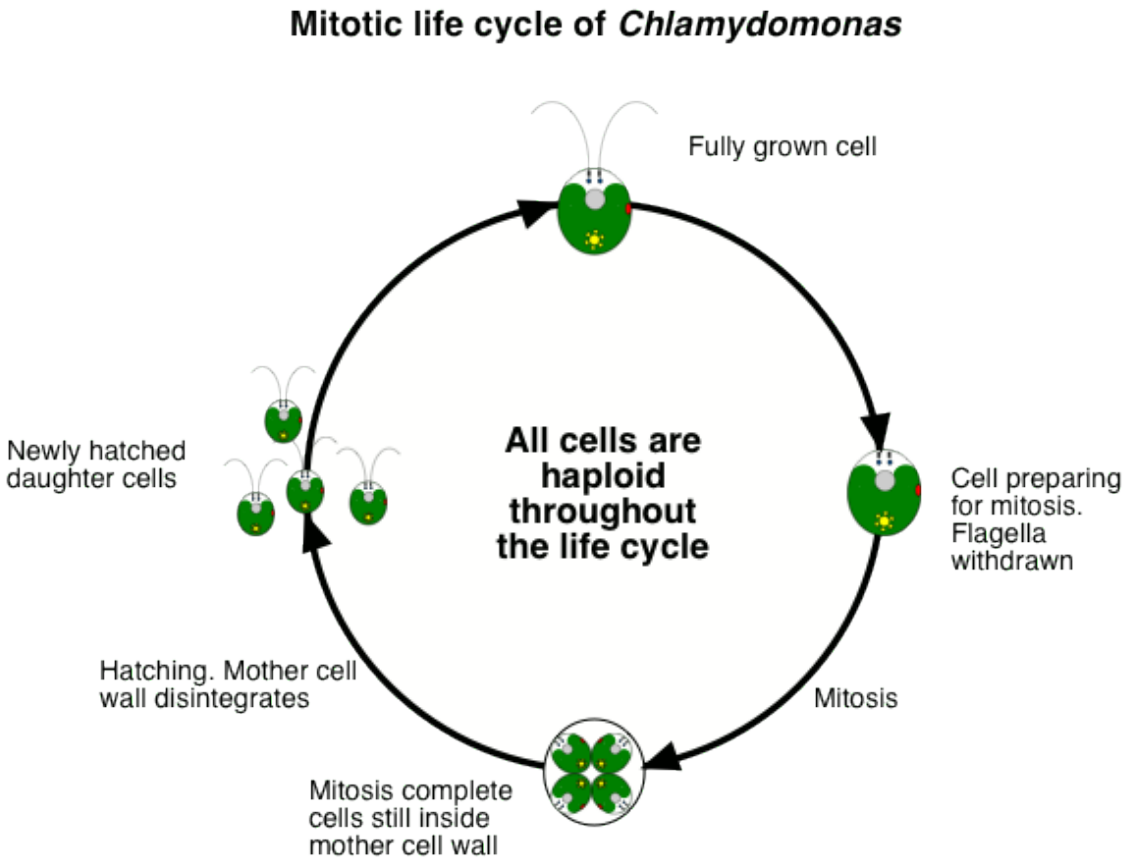

Figure 2. Vegetative (mitotic) life cycle of *Chlamydomonas*. Cells will stay in this mode as long as nitrogen levels are high. Under very good conditions the mother cell may divide into 8 or even 16 cells at each round.

However, under nitrogen deprivation the cells change their metabolism and stop dividing. These cells are now sexually active gametes and will mate with gametes of the opposite mating type (Fig. 3). Gametes will bind to each other using proteins on their flagella and after a short time the cells will form plus/minus pairs. These are pulled together by the flagella and an enzyme is released to shed the cell walls. Now the two cell membranes fuse to produce a single cell, the dikaryon. The dikaryon contains four flagella (quadriflagellate) but the nuclei and chloroplasts are from the two parents are still separate. Eventually the nuclei and chloroplasts from the two parents fuse to form a diploid cell, the zygote. The zygote develops a thick wall, which can protect the cell from adverse conditions for months. When conditions are better the zygote will undergo meiosis, the cell wall will lyse and four haploid offspring are released, two mating type plus, and two mating type minus.

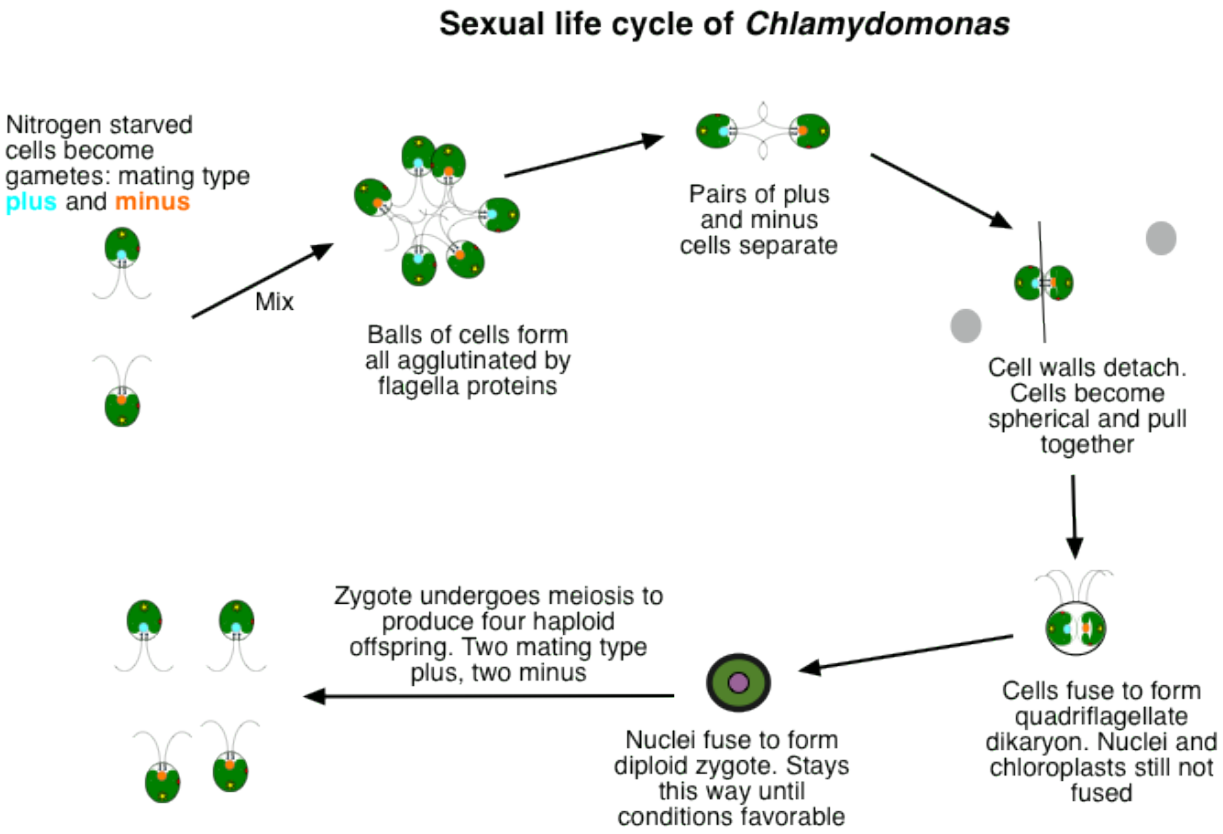

Figure 3. Sexual life cycle. In addition to the steps shown here a small percentage of zygotes may undergo mitosis, and produce vegetative diploid offspring.

Flagella Loss and Regrowth

The two flagella, each about 10 µm in length, have two functions. The first, as seen above, is to bring together cells of the opposite mating type in sexual reproduction. The second is to move the cells through the water. Swimming is generally achieved by a 'breast-stroke' type of action and can be seen at:

http://nutmeg.easternct.edu/~adams/ChlamyTeach/Swimming.htm

Chlamydomonas will shed its flagella under a variety of conditions. The reason for shedding flagella is not clear, though several suggestions have been made. A common idea is that it reduces the amount of membrane surface exposed to obnoxious chemicals. The downside of this idea is that it leaves the cell no way to escape from the environment.

General Appearance and Swimming Behavior

Place a drop of 620+ cells on a microscope slide and examine under 10x, 20x and 40x. Observe the swimming motion and the lack of interaction between cells. You may see some dividing cells: often the daughter cells remain inside the mother cell wall as a tetrad. Repeat the observations after mixing another sample of the cells with a drop of Protoslo, which will slow the swimming down enough to let you see the cells in more detail. Finally, add a drop of Lugol's iodine to the edge of the coverslip and touch a paper towel to the opposite side, to draw the iodine under the coverslip. This will kill the cells and stain the chloroplast. Again, look at the cells and try to identify the key features of the cells, such as the flagella, nucleus, eye spot and chloroplast, as diagrammed in Fig. 1.

Mating

Mix one ml of CC620+ gametes and 1 ml of 621- gametes in a test tube. Immediately place a drop on a slide and add a coverslip. Examine at 10x, 20x and 40x with and without phase contrast (if available). Initially you should see pairs and clumps of cells aggregating by their flagella. The adhesion interferes with swimming, leading to the characteristic jerky appearance of mating cells. This and the following stages are diagrammed in Fig. 2.

Loss of Cell Wall

After anywhere from a few seconds to a couple of minutes you should see the cell walls pop off and the cells will become more spherical in shape. Careful examination under 40x may show that the cell wall is in two parts, one large part and a smaller fragment from around the flagella. You may even be able to see the two holes in the cell wall where the flagella emerged. Soon after this the clumps should break up and pairs of cells (one+/one-) should become apparent.

Cell Fusion

Once the wall is gone, the cells are free to fuse. This occurs where the two are held together by the flagella. Initially a dumb-bell shape forms and then the two fuse completely into a spherical cell with four functional flagella. This quadriflagellate stage is motile, and lasts for several minutes up to an hour. Although the cytoplasm of the two cells have completely merged, the two nuclei are still separate, and this is termed the dikaryon stage.

Zygote Formation

The flagella resorb and a heavy cell wall starts to form around the cell. Internally the nuclei and chloroplasts from the two parents are fusing. This cell can remain in this stage for months, and be carried by wind or water to other locations.

Deflagellation and Regrowth of Flagella

Check that the cells are flagellated and swimming. Mix two drops of cells and a drop of Lugol's iodine and label the tube T0. The culture will be deflagellated by your instructor using either pH shock or mechanical shearing. Once complete, again take a drop and check that the cells are now motionless and have lost their flagella. At higher magnification you may even see the flagella floating around in the medium.

Regrowth Kinetics

Once the cells are deflagellated take a sample every 20 minutes and mix with Lugol's iodine, labeling the tubes T20, T40, T60, T80, T100. Estimate the length of the flagella in each sample as described by your instructor. Plot average flagella length against time. How does the rate of growth change over time?

Materials

- Wild type mating type plus cell, strain CC 620+. One plate of week-old culture on TAP medium.
- Wild type mating type plus cell, strain CC 621-. One plate of week-old culture on TAP medium.
- 10 mM HEPES pH 7.4, 500 ml
- Lugol's iodine, 50 ml
- 0.5 M acetic acid, 10 ml
- 0.5 M KOH, 10 ml
- TAP medium, 1 liter. Use half to make 1.5% agar plates.
- Glass homogenizer
- Blender (kitchen type is fine)
- 250 ml Erlenmeyer flasks (ideally with foam stoppers and plugged 1 ml pipettes inserted)
- Slides
- Cover slips
- Transfer pipettes
- Test tubes + racks
- Microscopes, preferably phase-contrast

Notes for the Instructor

To avoid making this paper too cumbersome I have not included a huge amount of material on the growth and maintenance of *Chlamydomonas*, nor on its structure, life cycle or on flagella structure and function. Much of the needed material can be found on the ***Chlamydomonas* Teaching Web Site**:

http://nutmeg.easternct.edu/~adams/ChlamyTeach/

in *The Chlamydomonas Sourcebook*, or in any basic text on cell biology. Likewise, many additional variations on these experiments, as well as more information on growth and working with *Chlamydomonas* are all on the same site.

Growth

The standard growth medium for *Chlamydomonas* is Tris-acetate phosphate (TAP). It is well buffered and the acetate allows the cells to grow rapidly (~10x per day). The required ingredients are simple to make and very stable. The exception to this is the Hutner's trace elements, which requires a tedious process. You can buy Hutner's trace elements from the *Chlamydomonas Center*, or fake it as described on the web site. Maintaining a few strains is not complicated. Growth on 1.5% agar plates on requires only monthly transfers and in many cases you can find a *Chlamydomonas* lab near you that will usually be happy to provide fresh strains if needed. Generally, I do not recommend commercially available strains as they have not been optimized for the experiments described here.

Mating

The keys to getting high mating efficiency are using strains that are very efficient, and then depleting the nitrogen while keeping the cells healthy in all other respects. Generally the best mating is obtained with the two wild type strains 620+ and 621-. Grow these on regular TAP plates under bright fluorescent light at room temperature for a week to ten days. On the day of the experiment, scrape the cells off the plate into about 10 ml of 10 mM HEPES pH 7.4. Pass the culture through a glass homogenizer, squeezing any clumps against the wall to flatten them. Once treated the volume can be increased to about 50 ml. You want a cell density of about 10^6 cells/ml for optimal results. Place the culture under bright light and either bubble with air or shake the flask. After 3-4 hours you can take a drop of each mating type and see how rapidly the mating types interact. It seldom takes much longer than this to get excellent mating. If you have an early morning class, the resuspension can be done the night before, but make sure there is enough liquid to allow for overnight evaporation from the bubbling.

Deflagellation

In the lab the commonest methods are using a blender or administering a pH shock. Other methods have been used, but will not be covered here.

Medium for Deflagellation

If you are interested in reflagellation then you want a medium that is good for the cells, even if it does not promote flagella integrity. In many cases this can be the regular TAP growth medium. Using a blender allows you to use almost any kind of medium, including TAP, but requires that the volume is large enough for the blender to work.

For pH shock, you will need a medium that has a low buffering capacity and contains Ca^{++}. The simplest is 10 mM HEPES (pH 7.4) with 1 mM Ca^{++}.

Mechanics of Deflagellation

Before starting it is always a good idea to take a drop of cells and check them under the microscope. Record the cell density and the percentage of flagellated cells.

Blender Method

Pour the cell suspension into a standard blender. Note the time. Add the cover and turn the machine as high as it will go for 30 seconds. Immediately take a drop and check for deflagellation under the microscope. If deflagellation is poor, blend for another 15 seconds. Over-blending may cause some cell lysis and reduce reflagellation. There is no need to pellet and resuspend the cells, unless the experiment itself needs it. The cells can be returned to their normal growth conditions and reflagellation will start almost immediately.

pH Shock Method

Place the beaker of cells into an ice bath and add a small magnetic stir bar. Place the ice bath on a stir plate and put the pH electrode into the beaker. Start stirring the suspension rapidly and add 0.5 M acetic acid dropwise until the pH is

4.5. Wait 45 seconds and then add 0.5 M KOH until the pH is back up to 7.4. Check for deflagellation and repeat if not satisfied. Note: This may reduce the percentage of cells capable of reflagellation and may also delay the onset of reflagellation. The cells may be resuspended in TAP with or without additives, depending on the experiment and allowed to reflagellate.

Measuring Reflagellation

Ideally students will measure the length of at least 20 flagella for each time point. Seeing flagella is not easy under regular brightfield, so phase contrast is much preferred. If phase contrast scopes are not available, treating the cells with Lugol's iodine helps make the flagella easier to see. Measuring the length can be done using a micrometer eyepiece, though this can be tedious, especially since flagella are curved. For faster results you can have students do a rough estimate of the length relative to the cell size (1/4, 1/2, etc), which will not be as accurate, but should show the kinetics of regrowth. My favorite way is to use a microscope camera to project the image onto a monitor, place a sheet of clear plastic on the screen and trace the flagella with a marking pen. This can be done very quickly and the sheet is removed and length of the flagella can be determined at leisure. If you want to know the absolute length of the flagella, use the same camera to project an item of known length onto the screen as a standard.

Two potential sources of error: some cells don't lose their flagella and others never regrow flagella. If students count these in their measurements the first case will result in artificially high values at the start and the latter in low numbers at the end. You can have students ignore any long flagella found in the first 30 minutes and any missing flagella after 30 minutes. Alternatively (not for the math-phobic students) you can get the total class numbers, estimate the frequency of both types at T0 and T100, subtract out each from the class data at each time point and then recalculate the average length.

Measuring Cell Density

Although these experiments do not need a precise knowledge of cell numbers, they can be readily determined using a hemacytometer. See the **Chlamydomonas Teaching Site** for details (Counting Chlamys).

Additional Experiments If There Is Time Or As Follow-Up Laboratory Exercises

Kinetics of the Mating Process

Mate the cells as described above. Every 15 minutes, take five drops of the mating culture and mix with a drop of Lugol's iodine in a fresh test tube. Put a drop under the microscope and estimate the percentage of cells unmated, clumped, paired off, fused, and as zygotes, at each time point and plot them over the next hour.

Mating Efficiency

Before mating, adjust the cell density of both mating types to the same value. Have students do a cell count before mating and then again at the end of the exercise. If every cell finds a mate then at the end of the lab, the total cell density will be halved. In fact some will not mate (never found a cell of the other mating type, is still a vegetative cell, etc.) so the reduction in cell density will be less than expected. You can either have them work out the efficiency from scratch or give them the formula:

% mating efficiency = 2 x % reduction in cell density.

So, if at the start they saw 100 cells/field of view and only 70 at the end, the reduction is 30% and the mating efficiency is 60%.

Complementation

This will require growing the two paralyzed mutant strains listed below. Mix CC 1032 pf 14^+ and CC 602 pf 1^- gametes as above. Examine under 20x phase contrast. Since both strains are paralyzed there is no jerking of paired cells, but you should be able see flagellar adhesion. Loss of cell wall and fusion should be similar to the first experiment. Examine samples at 15 minute intervals. Once the cells have fused and formed the dikaryon stage, check the quadriflagellates for motility. You should start to see cells swimming within 30 minutes at the latest. The two mutants are both missing a key protein needed for flagellar function, but each is missing a different protein. When the cells fuse, the protein missing from pf1 is supplied by pf14 and vice versa. As a result, both sets of flagella are now able to function again.

Effect of Cycloheximide on Reflagellation

Repeat the experiment but add 10 micrograms/ml cycloheximide to the cells immediately following deflagellation. Again, plot the regrowth over 80 minutes. How do the results look when compared with the original experiment? What might be responsible for the difference? If you used erythromycin, instead of cycloheximide, what results would you expect? Remember, cycloheximide blocks 80S (cytoplasmic) ribosome function, but not 70S (organelle) ribosomes and erythromycin has the opposite effect. Since flagellar proteins are made in the cytoplasm it has been observed that cells regenerate half-length flagella in the presence of cycloheximide. Presumably there is a pre-existing pool of flagellar proteins that allows this much growth. By contrast, erythromycin has no effect on the regeneration process.

Isolation of Flagella

If time permits, deflagellate cells as above. Spin the sample at 1000 x g for 10 minutes. Pour the supernatant into a fresh tube and repeat twice. Spin the third supernatant at 25,000 x g for one hour. Ideally you will have a small white pellet of pure flagella. In practice you will probably have

some green in the pellet due to cells not removed by the low speed spins. Look at the purified flagella under the microscope (phase contrast works best). If the flagella are solubilized in SDS-PAGE loading buffer and run on a 10% gel, you should be able to see a number of bands. Most prominent will be two bands at 55 kD that are the two tubulin subunits that make up about 80% of all flagella protein.

Acknowledgements

I would like to thank the 2009 ABLE conference organizers for the chance to present this workshop, and for the thoughtful and useful comments from the participants. I have tried to incorporate the suggestions made by the attendees.

Literature Cited

Stern, D. and E.H. Harris, G. Witman, Editors. 2008. *The Chlamydomonas Sourcebook,* 2nd Edition. Vol. 1-3 Academic Press, 2000 pages.

About the Author

Dr. Adams has worked with *Chlamydomonas* for over 30 years. His research has ranged from inheritance of chloroplast genes, to flagella structure and function, to the heat shock response in *Chlamydomonas*. Since coming to Eastern Connecticut State Dr. Adams has mentored over 50 independent study students who have worked on various aspects of the biology of *Chlamydomonas*.

Appendix

The diagrams can be downloaded from the **Chlamydomonas Teaching Site**:
http://nutmeg.easternct.edu/~adams/ChlamyTeach/

Mission, Review Process & Disclaimer

Citing This Article

Adams, M. 2011. Teaching with *Chlamydomonas*. Pages 237-244, in *Tested Studies for Laboratory Teaching,* Volume 32 (K. McMahon, Sr. Editor). Proceedings of the 32nd Conference of the Association for Biology Laboratory Education (ABLE), 445 pages. **http://www.ableweb.org/volumes/vol-32/?art=19**