Cell and Hybridoma Technology Course at the State University of New York College at Fredonia

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The Biology department at SUNY, College at Fredonia, enrolls about 300 Biology undergraduate majors and supports an MS graduate program of seven research students and ten in-service teachers. Our department offers students four different majors: Biology, Medical Technology, Recombinant Gene Technology, and Secondary Education. Cell and Hybridoma Technology is a team-taught spring semester course that usually enrolls about 15 students, who range from second semester juniors through Master's level graduate students. Because this course is a unique research-based course among our offerings, it most frequently attracts Recombinant Gene Technology majors, who most frequently enter graduate school.

There are several goals for this course including: mastery of aseptic technique, the initiation and maintenance of both animal and plant cell cultures, and the generation and use of monoclonal antibodies. This team-taught course provides students the opportunity to master skills in both animal and plant cell culture. In my portion of the course we study how to generate monoclonal antibodies directed against axolotl limb regenerate tissue. The plant cell culture portion of the course taught by my colleague exposes the students to the principles of callus formation, cloning, embryogenesis, and gene transfer. This brief narrative will detail only that portion of the course that deals with animal cell culture and the generation of monoclonal antibodies.

Our undergraduate educational system teaches many students to believe results of science (i.e., Research) experiments can be obtained in a two or three hour period. This perception is reinforced by the fact that as instructors we design weekly lab exercises that *are* completed in these short intervals—some students are under the impression that we must be able to obtain results in our own research in the same time interval.

To emphasize the time involved in research, students are involved in all aspects of the course (except ordering supplies). Each week a pair of students is given additional responsibilities assisting with the daily operation of the lab: preparation of media, washing and autoclaving glassware, cleanup of the lab, and procuring supplies from the stockroom. During the class they are responsible for daily observations of the cells. Constant participation by the students teaches them just how involved research really is, and they leave here better prepared for graduate positions and the expectations a mentor may have about work ethic.

Starting in late November I begin weekly immunizations of two mice with a homogenate consisting of ten, early cone-stage blastemata from regenerating axolotl limbs. Early immunizations with complete Freund's adjuvant are followed later by immunizations with incomplete or no adjuvant. Because I routinely interact with many of the students who are scheduled to take the class, a month or two before the class starts I invite some of them to become minimally involved in the preparation of the immunogen and to witness the injections. By the time of the final booster injection most if not all students have observed the immunization procedure. Once our fifteen week teaching semester begins, only four of my seven labs are scheduled lab periods, three at the beginning and one toward the end of the course. For the remainder of the time students participate in lab activities on their own time on a daily basis throughout the first half of the semester.

The lecture series follows the text by J. W. Goding, *Monoclonal Antibodies: Principles and Practice* (2nd ed. Academic Press). Because it is of paramount importance to the success of the course, the first week or two is devoted to an understanding of cell culture requirements and aseptic technique. Lectures involve media composition. buffering, and cell culture requirements. In lab each student is given a plate of NS-1 cells and expected to determine cell density on a daily basis, change the media every two or three days, and learn when to split cells. Any problems with aseptic technique can be corrected at this point. In an odd sort of way, I was actually disappointed this year because none of the students experienced contamination! (It is worrisome that they will not know what to observe or how to deal with bacterial or fungal contaminants.) Early repeti-

tive use of the hemocytometer and development of manual dexterity skills pay off as the course continues. By the midpoint in the course I am confident in virtually every student's skills and manipulation of the cultures.

During the second scheduled lab period I perform a demonstration fusion so that the students witness all the methods involved in the process. Because a good fusion depends in part on the smooth processing of the cells, expecting them to perform this the first time and base the whole class on their inexperience is a recipe for failure; the demonstration helps to overcome this limitation. I use standard polyethylene glycol fusion methods and subdivide the cells into about ten, 96-well culture plates at three different densities. The students take copious notes and get a feeling for the logistics of each step. They do this with earnest because they have been told that next week they will repeat this process with pairs of students responsible for different steps in the fusion process.

The third lab is a fusion performed by the student with my acting in an observer and consultant role. Students prepare all solutions ahead of time, ensure that the myeloma cells are actively growing, and split the cells 24 hours prior to fusion, so that they get the feeling for the necessary set up. (Yes, a headache for me, but why should I make this look easy by having everything ready for them?) It is interesting to watch the group dynamic in action. No one student attempts to take over and "do it all," and, to ensure the cells are not contaminated by someone with poor sterile technique, most students watch intently and correct their fellow students' methods. When the student fusion is complete, each student receives one 96-well plate of cells that contains three different densities of cells, and it is that student's responsibility to maintain and test these cells for the rest of the class.

Because a successful fusion depends in part on smooth processing of the cells, the completion of two fusions increases the likelihood that one of the procedures will produce viable hybrids for use by the class. At this point in the procedure we are at week five (of fifteen) of the class, and in lecture I have introduced cell culture, the theory of monoclonal antibodies, and the theory behind the production of hybridomas. In the coming lectures we discuss HAT treatment to select for hybridomas and different screening assays that can be used to detect the presence of antibody producing cells.

From this point on the advantage/disadvantage of the course is that I only hold two or three more scheduled labs: one in the middle to demonstrate cloning and one at the end to do both an ammonium sulfate precipitation of the antibody and a dot and/or Western blot. The advantage for both the students and myself is that there are no scheduled labs to prepare for the next few weeks. Students are responsible for daily observation and treatment of their cells: HAT treatment, identification of hybridomas, testing the culture medium, freezing sample cells, and cloning at least one cell sample. The disadvantage is that I need to be available when students working on their own time need assistance, or new media needs to be prepared.

Students identify productive hybridoma cultures using indirect immunofluorescence of cryostat sections that they have prepared themselves. This means that the students must learn the basics of axolotl limb regeneration, freezing of tissues, cryostat sectioning, and immunofluorescence. After amputating limbs they watch observable regeneration and freeze a pair of limbs at the mid-cone stage of regeneration. They continue to observe regeneration in the re-amputated limbs so that they can witness the entire process. We discuss the stages of regeneration, the controls over this process (nerves, hormones, and wound epithelium). They quickly recognize that the immunogen is composed of a complex mixture of molecules and that the antibodies produced by this immunization protocol will be different for each set of hybridomas that they are examining. They learn the process of cryostat sectioning and indirect immunofluorescence. Initially, each step needs to be monitored carefully, but as they continue to work with the material their expertise and confidence levels increase. Once antibody producing hybridomas have been found, the cells are transferred to larger plates, grown, and samples of the cells are frozen for later study. The students then learn to clone the cells by limiting dilution and to precipitate and concentrate antibody from the about 25 ml. of culture medium using ammonium sulfate precipitation and dialysis. Time permitting, we also use the antibody in a dot blot analysis of various tissue samples to show how an ELISA is performed. Finally, we discuss how an antibody might be used to screen a cDNA library to identify the gene responsible for the production of the antibody. Toward the end of the course each graduate student in the course is

responsible for presenting a seminar that demonstrates how monoclonal antibodies have been used in different research projects.

While the course is very time consuming both for myself and the students, I feel that the daily involvement of the students in the course is an experience that they enjoy and a challenge that they rise to meet. They enter the course with anxious anticipation, knowing it will mean a large time commitment, but most of them leave the course knowing that they learned first hand how to really do cell cultures, and that the time commitment was not all that bad; In fact there are some students who do not want the class to stop and want to continue working on their cells after the course has finished. The students leave the course with cell culture experience and both a better understanding of what a monoclonal antibody is and a taste for how much time research involves.