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Research Techniques in Molecular Biology

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Extended Abstract

Genetics 420 (*Research Techniques in Molecular Biology*) is a new fourth-year laboratory course at MacEwan University that couples the acquisition of fundamental competencies with the opportunity to develop critical research skills. These fundamental competencies include a foundation of knowledge, exposure to common methodologies, well-developed communication and information literacy skills and the ability to critically examine scientific data. In addition, the opportunity to evaluate, integrate, and apply information to the design and management of experiments, troubleshoot experimental difficulties and discuss experimental outcomes is valuable, particularly for students interested in pursuing opportunities in research.

MacEwan University is a learner-focused undergraduate university. Genetics 420 is a capstone course in the Molecular Genetics stream of the Biology degree program, which can be taken by students that have completed two second and two third-year courses in genetics and molecular biology. Students attend two 3-hour lab periods each week in both fall and winter terms for a total of 120 instructional hours. These senior undergraduate students are mentored in a small class setting with a maximum enrollment of 12 students, in which each student works independently to conduct his or her experiments. In addition to bench work, each lab period includes discussions of theory related to the techniques being employed, as well as information literacy and scientific writing. This course therefore develops both scientific literacy and technical skills, and prepares students for future graduate studies or research work.

The fall semester project,*Cloning, mutagenesis and expression of enhanced green fluorescent protein (EGFP)*, focuses on techniques used to create recombinant DNA constructs for the expression and analysis of proteins. Students are provided with a source plasmid containing an enhanced green fluorescent protein (EGFP) gene, which is cloned into a pET15b expression vector using directional cloning and ligation, allowing the EGFP to be expressed with a histidine tag. Students review the extensive literature on GFP structure, each design their own mutation, and predict how it will alter EGFP function. The plasmids they have built serve as templates for QuikChange site-directed mutagenesis, using a method adapted from Moffet (2009), to create their mutant gene sequences. Sequencing, which is performed by students in-house,is used to confirm the presence of the desired mutation. Students then express their mutant EGFP proteins in *E. coli* and purify them by nickel-affinity chromatography. The proteins are quantified, examined by SDS-PAGE, and their fluorescent properties are characterized by spectrofluorophotometry and fluorescence microscopy. Each mutant protein can then be compared to the wild type EGFP. In previous years, this procedure has allowed students to create mutated versions of EGFP with shifted excitation and emission spectra, increased brightness, and decreased photobleaching.

The winter project, "β-globin expression in a human chronic myelogenous leukemia (CML) cell line", focuses on cell culture and the analysis of changes in gene expression. K562 is a human CML cell line known to differentiate into fetal hemoglobin producing cells in response to treatment with a variety of chemicals. In addition, differentiation is induced by glutamine deprivation. Students independently review previous research and each student submits a short proposal identifying a chemical to induce differentiation. Following instructor approval, the students independently culture cells, testing their chemical alone and in combination with glutamine deprivation. Cell proliferation is monitored and differentiation is quantified using a benzidine staining assay. Students observe a decrease in proliferation in response to differentiation. Students then extract RNA and protein from their cells in order to compare β-globin expression in four treatments: control (+glutamine, –chemical); –glutamine; +chemical, –glutamine; and +chemical. Western analysis and northern analysis are then conducted to compare protein and mRNA expression, respectively. In addition, RT-PCR followed by semi-quantitative real-time PCR was performed (Hancock *et al.*, 2010). In the past, glutamine deprivation has effectively induced differentiation, reduced cell proliferation and resulted in detectable changes in β–globin expression, while the effects of different chemicals have been more variable.

This course, in addition to giving students practical lab experience, aims to develop skills in information literacy, scientific writing, problem solving and critical thinking. Many opportunities for both summative and formative assessment are provided throughout the course with a variety of assessments and scaffolded assignments. The fall term assignment introduces students to two common forms of writing – the research proposal and the review paper. Students conduct a literature review of review articles to familiarize themselves with research on GFP structure and function. Based on the structural information they learn, students write a research proposal in which they describe the theoretical basis for their mutation, hypothesize an outcome based on the literature, and describe the experimental methods to be used to create the mutation. The students then conduct a literature review of primary literature based on information learned while preparing the proposal. First, a detailed outline is submitted, in which students describe all literature sources to be used to write a literature review on GFP, and outline how the information will be used and organized. Second, students submit a draft that is evaluated for content, organization, and skills of scientific writing. Lastly, students hand in a polished literature review describing the use of GFP in research, and emphasizing the link between its structure and function as a fluorescent marker. In the winter term, a second scaffolded assignment is completed in the form of a formal scientific lab report. Students first hand in an annotated bibliography identifying key resources for their full lab report. These sources are summarized and their relevance to the lab report is clearly defined. In addition to the annotated bibliography, opportunities for summative feedback are provided through submission of an introduction outline, followed by a draft for which feedback is given and a small grade assigned. Students also have the opportunity to hand in the materials and methods, figures and results sections for a specific experiment for summative feedback, though this component is optional and no grade is assigned.

Throughout both terms, students maintain a complete and up-to-date lab notebook. This notebook contains an explanation of the purpose and theory of each experiment; predictions of expected results, based on background theory; a complete and detailed outline of the experimental procedure; and a description of the results, accompanied by analysis and interpretation. Use of these lab notebooks ensures that students are prepared prior to each lab period, increasing engagement in the lab exercises. It also increases student understanding of the labs by making them consider the reasons why each procedure is used and how the results are interpreted. Lastly, it introduces students to best practices followed by many research and development labs. As a final summative assessment, two end-of-term examinations are given to assess acquisition of the theoretical knowledge underlying the methods used in the lab, and the ability to apply this knowledge critically to new experimental situations.

This course provides an excellent opportunity for students to develop a variety of skills needed for a career science. The progress made by students in this course demonstrates its success – by the end of the winter project, students are able to work independently and thoughtfully analyze their results. Student feedback has indicated that students feel this has been one of the most valuable course experiences of their degrees.

Keywords: molecular biology, undergraduate research projects

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