Research Techniques in Molecular Biology

MacEwan
Grant MacEwan University

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Introduction

Grant MacEwan University is an undergraduate university offering a BSc degree in which students may major or minor in Biological Sciences. Research Techniques in Molecular Biology (Genetics 420) is a fourth-year laboratory course offered for the first time in 2011-2012. Students attend two 3-hour lab periods each week in both the 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 addition to bench work, each lab period includes discussions of theory related to techniques as well as information literacy and scientific writing. Critical thinking and problem solving are emphasized. This is an academically rigorous course that develops both scientific literacy and technical skills.

Learning Objectives

At the end of this course, students should be able to …

- Perform a wide variety of methodologies commonly used in molecular genetic analyses and discuss the principles underlying techniques that have been employed
- Keep a comprehensive laboratory notebook outlining experimental setup, protocol details and data collected
- Analyze experimentally collected data
- Evaluate, integrate, and apply information to plan experiments, troubleshoot experimental difficulties and discuss experimental outcomes
- Effectively communicate scientific ideas orally and in written form

Fall Project: Cloning, Mutagenesis and Purification of EGFP

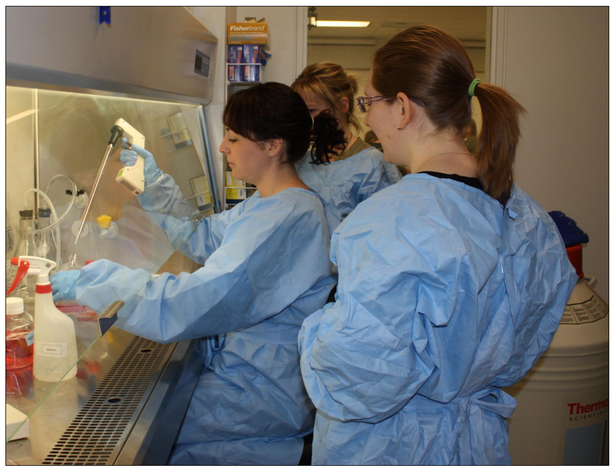
This project focuses on techniques used to create recombinant DNA constructs for the expression and analysis of proteins. Each student proposes a specific mutation to an enhanced green fluorescent protein (EGFP) and predicts how it will alter the proteins function, using the existing literature. EGFP is cloned into an expression vector then mutated using site-directed mutagenesis. The mutated protein is then expressed, quantified, characterized and compared to the wild type EGFP.

Techniques – Fall

- Primer design
- PCR amplification
- Cloning and transformation
- DNA extraction and manipulation
- Site-directed mutagenesis
- Gel electrophoresis – protein and DNA
- DNA sequencing
- Protein tagging and recombinant expression
- Protein purification and quantification
- Spectrofluorophotometry
- Fluorescence microscopy

Winter Project: γ-Globin Expression in K562 Cells

This project focuses on methods to analyse gene expression. K562 cells can be induced to differentiate into hemoglobin producing cells. Students review the literature to identify chemicals known to induce differentiation. Students independently culture cells, testing their chemical alone and in combination with glutamine deprivation. Cell proliferation is monitored, differentiation is quantified, and γ-globin expression is observed.



Techniques – Winter

- Cell culture and manipulation
- Differential cell staining
- Solution preparation
- RNA and protein extraction and quantification
- RT-PCR
- Real-time PCR
- Northern analysis
- Western analysis

Assessments of Learning

Both summative and formative assessment are utilized with a variety of assessments and scaffolded assignments. All written assignments are assessed using detailed rubrics which are provided to students in advance.

Students are not currently directly evaluated on their technical skills. We are currently investigating strategies to evaluate these skills and hope to incorporate this in the near future.

Examinations – Fall & Winter

- Written examination; short and medium answer questions
- Emphasizes critical thinking and problem solving skills by asking students to apply knowledge to new experimental situations
- Assesses acquisition of theoretical knowledge

Research Proposal – Fall

- Includes a literature review
- Based on structural information, students design mutations to alter the function of GFP
- Written proposal describes the theoretical basis for the mutation, hypothesizes an outcome, and outlines the experimental methods

Literature Review – Fall

- Builds on the research proposal
- Describes the use of GFP in research, emphasizing the link between its structure and function as a fluorescent marker
- First, a detailed outline is evaluated
- Second, a draft is evaluated
- The final literature review is submitted at the end of the fall semester

Research Proposal – Winter

- Includes a brief literature review to provide rationale for the proposed chemical inducer
- Identifies the catalog number and an assessment of chemical affordability and availability using the manufacturer website
- Provides a Health & Safety summary using the MSDS
- Includes a protocol for making a stock solution

Lab Report – Winter

- First, an annotated bibliography is submitted and evaluated
- Second, individual sections are submitted periodically for summative feedback
- The full lab report is submitted at the end of the winter semester

Laboratory Notebook – Fall & Winter

- Includes:
  - Explanation of the purpose and theory of the experiment
  - Predictions of expected results, based on theory
  - Complete details experimental procedures
  - Results, accompanied by analysis and interpretation
- Checked at the beginning of each lab to ensure students are prepared
- Assessed at the end of each semester

Conclusions

This course provides an excellent opportunity for students to develop a variety of skills needed for a career in 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.

References

Hancock, D., Funnell, A., Jack, B., Johnston, J. 2010. Introducing undergraduate students to real-time PCR. *Biochemistry and Molecular Biology Education* 38: 309-316.

Moffet, D. 2009. From gene mutation to protein characterization. *Biochemistry and Molecular Biology Education* 37: 110-115.