

Course Description

BCMB 406B is a project-based course that will build on research skills you have developed in previous lab courses. Unlike other lab courses that consist of several distinct labs, 406B has three labs that build on each other to create a continuous research project from start to finish. The overall aim of 406B is to create and characterize a mutant carbohydrate binding module (CBM). CBMs are accessory modules of glycoside hydrolases (GH) which are enzymes that hydrolyse the glycosidic bond between carbohydrates. As the name suggests, the CBM targets the enzyme to its substrate by binding to carbohydrates.

In lab 1, you will learn the principles of primer design and use a variety of web-based tools to design and evaluate a set of primers for site directed mutagenesis. The newly created mutant CBM construct into an expression host.

Lab 3 focuses on the purification and characterization of the mutant protein. Initially, you will induce expression of the mutant CBM protein and purify the protein using Immobilized Metal Affinity Chromatography (IMAC). Once purified, you will assess the mutant CBM's ability to bind carbohydrate using two techniques that will allow you to compare the function of the mutant CBM to that of wild type. Finally, you will attempt to crystallize the mutant protein and use modelling software to compare and contrast the structures of the mutant and wild type CBMs.

In this course, emphasis is placed on experimental design, data analysis and problem solving with the intention of developing your ability to work independently in the lab.

Intended Learning Objectives for 406B

Upon completion of 406B you will be able to:

- x Describe the theory and principles of primer design, site-directed mutagenesis and protein expression, purification and characterization
- x Develop proficiency in practical skills and in silico techniques used for primer design, site-directed mutagenesis and protein expression, purification and characterization
- x Solve typical calculations used in biochemistry and microbiology experiments
- x Evaluate experimental controls
- x Generate a written record of data in a lab journal
- x Evaluate data generated and summarize findings in written lab reports
- x Compare and contrast data generated in the laboratory with that of relevant published research articles

Week	Dates	Lab	Day 1 (5 hours)	Day 2 (2 hours)	Due Dates
1	Jan 11-15	Lab 1 : Primer Design	Primer design and evaluation using web-based tools		
2	Jan 18-22	Lab 2: Site Directed Mutagenesis of CBM Proteins	In silico cloning		Day 2: Lab 1 Report
3	Jan 25-29		Inverse PCR, agarose gels, DpnI digestion, electrocompetent cells	Electroporation	
4	Feb 1-5				

Evaluation and Assessment

Percentage Breakdown for the Course:

Exams	40 %
Lab Reports	30 %
Practical Assessment	20 %
Laboratory Journal	10 %

UVic Grading Scheme

A ⁺	90 - 100	B ⁺	77 - 79	C ⁺	65 - 69	F	< 50
A	85 - 89	B	73 - 76	C	60 - 64	N **	< 50
A ⁻	80 - 84						

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