BIO567L - Biochemistry, Cell & Molecular Biology Laboratory II
Spring 2015

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Monday and Wednesday
1:00 - 3:40 pm - Section 1
4:00 - 6:40 pm - Section 2

Class Objectives
This course is designed to teach molecular and cell biology techniques commonly encountered in graduate school and biotech. The class is divided into two parts. The first section is more supervised. The second section is much more independent research. Initially, you will isolate DNA and Taq DNA Polymerase in order to perform a 16S rDNA PCR with proper controls. You will then use these techniques to characterize a bacterial community or unknown isolate by sequencing. Finally, you will characterize, clone, sequence and express a BAM (Bacteriophage Adherence to Mucus) domain into T7 phage. In the second part of the class, you design and conduct your own experiments using the modified phage. If already in research labs, students will be able to continue applicable research and have it count towards this course. Other students will be involved in projects contributing to the further development of the BAM viral-prey interactions model. Unlike other laboratory courses, you will be responsible for making solutions, troubleshooting protocols, and interpreting the results. You will work in groups of 2, but you are expected to develop your own skills.

Section 1
Characterization of an unknown Bacteria or bacterial community by 16S rDNA cloning and sequencing
1 - Isolate bacteria or total DNA from environmental sample
2 - PCR 16S rDNA amplification
3 - Clone with TOPO kit
4 - Sequence with 27F
5 - Bioinformatic analyses of sequences
6 - Production of *Taq* DNA polymerase

Cloning of Bacteriophage Adherence to Mucus (BAM) domains, Virulence Factors (VFs), and Viral-Encoded Caspases (V-Caspases)
1 - DNAs from mucus layers (possible environmental sequencing)
2 - Bioinformatics to predict BAM domains, VFs, V-Caspase sequences and design appropriate PCR primers
3 - T7 cloning

Section 1 Practical (possible examples, you need to know how to do everything in the first section)
Calibration of pipettors
Solution preparations
Autoclave and plate pouring
PCR
Agarose gel electrophoresis
TOPO-cloning
Bioinformatic analyses of 16S rDNAs

Section 2 - Design and conduct independent research.
Possible research questions/goals:
1) Metagenomics and cloning of novel BAM genes from other surfaces
2) Measure binding of T7-BAM phage to different polysaccharides (e.g., in aquariums, tissue culture, etc)
3) Consumption of *E. coli* transformed or transduced (plasmid or T7 vector) with viral-encoded VFs.
4) Determine the phage and viruses associated with a wine fermentation.
5) Your ideas?

Required Materials:
Lab notebook – a normal compositions book will be fine
Lab coat, Fine-tip Sharpie, Calculator
Computer or smart phone with internet access

Laboratory Notebooks:
Notebook Table of Contents
Experiment title, date, and page numbers

Title
State the title of the experiment.

Purpose
State the purpose of the experiment. Why are you doing the experiment?

Data/Calculations/Results
Include all data: figures, tables, calculations, etc. Introduce each result section.

Discussion/Conclusions
State any conclusions you have regarding data and why or why not expected results were obtained. Example: As expected, relatively pure, undegraded RNA was isolated from

mammalian cells as seen in Figure 1, Lanes 1-3, which represent 1, 5 and 10μL of mammalian RNA, respectively.

Tape protocols, disks with data, Post-It notes into your notebook. The main purpose of the notebook is to be understandable at a later date....

Grading

Lab notebooks
Laboratory notebooks will be periodically checked as part of your subjective grade (50 points total). These notebooks can also be used during the practical exams, so it is to your advantage to do a good job.

Lecture Questions, short write ups, pop quizzes and weekly reports for Independent Research section
Lectures are given in a very interactive manner. Students are expected to be able to answer questions about the subject being addressed that day. Pop quizzes and short write ups will be given throughout the semester on material already covered and also on new material that you should have read before coming to class (up to 200 points).

Practical
There will be one practical worth 50 points.

Presentation of Final Project
Ten minute PowerPoint presentation of your independent project (100 points).

Grading Scale

95 - 100%	A
90 - 94%	A-
84 - 89%	B+
80 - 83%	B
77 - 79%	B-
74 - 76%	C+
70 - 73%	C
67 - 69%	C-
64 - 66%	D+
60 - 63%	D
57 – 59%	D-

Attendance
You must attend each class meeting. If you miss a class, let me know why and when. If you miss 2 classes you will receive an incomplete for the course.

Plagiarism Policy
Plagiarism will result in an "F(ail)" for the course and the student will be reported to the Students Rights and Responsibilities Center. If a student wants to protest or cases where it is not clear who is the plagiarist, then the case will be handled by the Students Rights and Responsibilities Center. Until the case(s) is resolved, an “I(ncomplete)” will be issued.

Schedule for Fall 2015 (Mondays and Wednesdays)

Date **Required Preparation, Tasks (come to class familiar with protocols to be completed), and Assessment**

August

Aug 24th (M) **Entrance Exam**
Reading - BAM, T7 cloning, DNA isolation
Tasks - making LB plates with antibiotics

Aug 26th (W) **DNA Isolation Lecture**
Check in
Tasks - Autoclave, pour plates with antibiotics, read about DNA isolation
FLR gone pm session

Aug 31st (M) **16S rDNA PCR Lecture**
Bring a sample (soil, teeth plaque, bark, skin, water, etc. - be creative) to class for your DNA and/or bacteria isolations
Reading - Read and understand MoBio Soil DNA Isolation protocol
Tasks - Pipetting, calibrations, making solutions, dilution problems and Soil DNA isolation
Quiz - DNA Extraction

September

Sept 2nd (W) **PCR and Electrophoresis Lecture (reagents, troubleshooting)**
Tasks - set up 1st PCR using DNA extracted from your sample.
Quiz - PCR

Sept 7th (M) Labour Day Holiday

Sept 9th (W) **TOPO Cloning lecture**
Tasks - Electrophoresis of PCR, clone into TOPO

Sept 14th (M) **Protein Isolation Lecture**
Tasks - Taq DNA Polymerase protein isolation
Quiz - Cloning

Sept 16th (W) **Tasks** - PCR with own Taq & prep TOPO clones for sequencing
Send out PCR products for sequencing as soon as you have them
Quiz - Protein Isolation

Sept 21st (M) **BAM Lecture (Barr), VF and V-Caspase Lecture (Ben) and Bioinformatics on BAM Domains (Sean)**
Quiz - BAM model, Virulence Factors, Caspases, Tranduction

Sept 23rd (W) **Tasks** - Design BAM, VF, or V-Caspase primers and order (some people will probably just have genes synthesized)
Quiz - Primer Design
FLR gone

Sept 28th (M) **Tasks** - BAM, VF, V-Caspase cloning into T7

Write up of 16S rDNA results is due Sept 28th

Sept 30th (W) **Tasks** - BAM, VF, V-Caspase cloning into T7

October

Oct 5th (M) **Tasks** - BAM, VF, V-Caspase cloning into T7

Oct 7th (W) **Section 1 Practical**

Tasks - Discuss preliminary ideas for independent projects with Ben and Forest

Oct 12th (M) **Discussions of Independent Projects**

Tasks - Each group needs to make a 10-minute presentation about their independent project

Oct 14th (W) to Dec 2nd (W) Independent Research

During this time each group needs to email both Ben and I weekly updates

FLR gone Oct 14th (W)

Nov 11th (W) Veteran's Day Holiday

FINAL PROJECT PRESENTATIONS Dec 7th and 9th

Last Day in Lab is Dec 2nd (W)

All reports and Tasks are due by Dec 9th