CM505 NUCLEIC ACIDS FOR NON-LIFE SCIENTISTS

Instructors:

Coordinator: Carol Wilusz, Associate Professor MIP, CMB

Required Texts and Materials:

No required texts. Students will require access to LabArchives Electronic Lab Notebook which can be purchased for $15 per semester.

Description:

Graduate students from both biological and computational/mathematical fields will be trained in basic molecular biology including nucleic acid structure, function and manipulation. Students will gain hands on experience in the common techniques used to quantify, quality control and manipulate nucleic acids.

Objectives:

Students will be able to:

- Utilize an Electronic Laboratory Notebook to describe experimental approaches and results.
- Safely and effectively use the equipment found in a standard molecular biology lab.
- Isolate, quantify and quality control nucleic acids from prokaryotes and eukaryotes.
- List the primary differences between prokaryotic and eukaryotic nucleic acids and their gene structures.
- Perform reverse transcription and polymerase chain reaction (PCR) to amplify sequences of interest from total RNA.
- Describe the steps of PCR and how they should be adjusted for specific applications.
- Explain how various commercial enzymes are used to analyze and manipulate nucleic acids.
- Describe how hybridization can be used to select, detect and quantify specific nucleic acid sequences.
- Interpret results of DNA sequence analysis and identify differences between predicted and actual sequences.

Pre-requisites:

No prerequisites – this class is designed for non-majors with graduate standing.

Credit Hours: 1 Credit

Class Breakdown:

This is a 1CR lab class offered over 4 weeks with 8 hr per week of contact time. This course is designed to require an average of 4 hours per week of outside work (preparation, problem sheets, reading, online presentations, quizzes etc). Students with weaker biology backgrounds may need to spend more time preparing for class and/or completing homework.

Teaching Strategies:

Labs, in class presentations, quizzes, online presentations, class discussion/problem solving activities.

Evaluation:

Laboratory Notebook	50%
Online Quizzes	10%
Problem Sets	15%
Attendance/Participation	5%
Final Exam	20%
TOTAL	100%

Grading Scale:

A	90-100
B	80-89
C	70-79
D	60-69
F	59 & below

This class does not issue grades of + or –

Courses with Overlapping Content:

CM502: Techniques in Cell & Molecular Biology – 3 credit course offered to 1st year CMB students and other life sciences graduate students. Not appropriate for non-majors and has a large amount of non-nucleic acid content.

BIOM533: Biomolecular Tools for Engineers – 3 credit course offered to Biomedical Engineers. Has a large amount of material not relevant to nucleic acids (e.g. live cell imaging, single molecule optics)

MIP611: Advanced Microbiological Research Methods – 4 credits, open only to Microbiology MSB students and has a large amount of material not relevant to nucleic acids (western blots, cell culture etc).

WEEK 1

DAY 1

PRESENTATION: Introduction to CM581A1 (30 min)
Overview, objectives, expectations, grading, academic integrity
Introduction to LabArchives Electronic Lab Notebook (20 min)

LAB: Introduction to the Molecular Biology Laboratory (1 hr 40 min)
Pipettes, Gels, Centrifugation, Organic Extractions, Ethanol Precipitation, Nanodrop, Enzymes
Includes tour of lab and practice with equipment

HOMEWORK: Quiz 1 (30 min)
LabArchives Set Up and read protocol (1 hr)
What is DNA? (40 min online videos)
Central Dogma of Molecular Biology – DNA->RNA->Protein
What is a gene? A genome? A chromosome? Extrachromosomal Elements?
- nucleotides
- base-pairing/structure
- chromatin
- methylation

DAY 2

PRESENTATION: Isolation and Quantification of Nucleic Acids (50 min)

LAB: Isolation of Genomic DNA from unknown bacteria (2-3 hr with incubations)
DNA Isolation, Nanodrop to assess concentration. Restriction Digestion overnight.

HOMEWORK: LabArchives Record Keeping and Read Protocol (30 min)
Quiz 2 (30 min)
Agarose Gel Electrophoresis, Markers, Nucleic Acid Stains (30 min online video)

WEEK 2

DAY 1

LAB: Gel Electrophoresis and Imaging (1 hr)

PRESENTATION: Enzymes to make, repair and destroy DNA (50 min)
What are enzymes? What do they need to work?
Replication (DNA-dependent DNA polymerases)
Endonucleases (Restriction Enzymes), DNAses
Ligases and DNA repair

PRACTICE: Problem Solving Exercise (1 hr 30 min)

HOMEWORK: Genome Variation (50 min online video)
SNPs/Indels/VNTRs
Repetitive Elements - Transposons/Microsatellites
LabArchives Record Keeping and Read Protocol (30 min)
Quiz 3 (30 min)

DAY 2

PRESENTATION: The Polymerase Chain Reaction (50 min)
- Choosing the right enzyme
- Optimizing the reaction conditions
- Visualizing the products
- qPCR

PRACTICE: Problem Solving Exercise (50 min)

LAB: 16S rDNA amplification via PCR; Gel Electrophoresis, cloning

HOMEWORK: LabArchives Record Keeping and Read Protocol (30 min)
Quiz 4 (30 min)
Sequencing (Online video 30 min)

WEEK 3

DAY 1

LAB: Sequence Analysis (50 min)

PRACTICE: Problem Solving Exercises (1 hr 30 min)

HOMEWORK: What is RNA? (online lecture, 50 min)
nucleosides
base-pairing
structure
- cap, poly(A)

different types of RNA
- functions
- unique properties

DAY 2

LAB: RNA electrophoresis, reverse transcription, PCR

PRESENTATION: Enzymes for making, modifying, and degrading RNAs
Transcription (DNA-dependent RNA polymerases)
Reverse Transcription (RNA-dependent DNA Polymerase)
Ribonucleases, Dicers etc
Poly(A), Poly(U) Polymerases
Capping/Decapping Enzymes
PseudoUridylation, Methylation etc

PRACTICE: Problem Set 1 (30 min)

HOMEWORK: LabArchives Record Keeping and Read Protocol (30 min)
Detecting Nucleic Acids (online video 50 min)
Modified nucleotides
- Fluors, ^{32}P, 4-thioUridine, Biotin

Probes and Primers
Hybridization
- Blotting

Microarrays

Quiz 5 (30 min)

WEEK 4

DAY 1

LAB: Gel electrophoresis to visualize RT-PCR (50 min)

PRESENTATION: Gene structure and function (50 min)

Prokaryotic gene structure and function
- Promoters
- Terminators
- Operons
- Small RNAs

Eukaryotic gene structure and function
- Promoters
- Introns and splicing
- UTRs
- Polyadenylation signals
- Chromatin

HOMEWORK: LabArchives Record Keeping (30 min)

Prepare for Final Exam – practice provided

DAY 2

FINAL EXAM

COURSE SURVEYS