BIOCHEMISTRY/MOLECULAR BIOLOGY @ CCHS 2016-2017

1st UNIT – Biochemistry

I. Basic Lab Techniques

a. **LAB: Measurements, Micropipetting, and Sterile Techniques**
   i. practice using micropipets, pipets, and an ultracentrifuge

II. Amino Acid and Protein Structure

a. Basic Chemistry Review
   i. Lecture – Basics of Chemistry
   ii. Lecture – Water, Buffers, and pH
   iii. Lecture – Henderson/Hasselbalch
   iv. Lecture – Principles of Gel Electrophoresis

b. **LAB: Gel Electrophoresis**
   i. preparation of various concentrations of agarose gels/buffer systems
   ii. casting, loading, and running gels under various conditions

c. Introduction to Organic Chemistry
   i. Lecture – Carbon Skeletons and Amino Acids
   ii. Lecture – Protein Structure: Primary → Secondary → Tertiary → Quartenary
   iii. Lecture – Ionization of Amino Acids
   iv. Protein Function
      1. myoglobin, hemoglobin, cytochrome c, albumin
      2. GFP and the fluorescent proteins (intro for later…)

d. **LAB: Isoelectric Focusing of Proteins via Gel Electrophoresis**
   i. predict outcome of electrophoresis based on different buffer systems
   ii. staining/destaining techniques with Coomassie Blue
   iii. native vs. non-native SDS PAGE

e. **LAB: Using a Spectrophotometer**
   i. prepare a standard curve of the biuret reaction from scratch
   ii. use the standard curve to then determine the concentration of multiple unknown samples
   iii. practice with Excel to interpret results

III. Enzymes

a. Enzymes
   i. Lecture – General Properties
   ii. Lecture – Factors Affecting Enzyme Action

b. Enzyme Kinetics, Inhibition, Regulation
   i. Michaelis-Menten equation
   ii. v_{max}, k_{cat}
   iii. Lineweaver-Burk (double-reciprocal plot)
   iv. competitive and noncompetitive inhibition

c. **LAB: Enzyme Kinetics**
   i. predict relative reaction rates on multiple runs with various enzyme and substrate concentrations; coupled with use of the Spec-20
   ii. determine nature of inhibition upon data analysis

2nd UNIT – Molecular Biology

IV. DNA History, Structure, and Function

a. Deoxyribonucleic Acid
   i. Lecture – History of DNA
   ii. Lecture – DNA Replication/Amplification/Sequencing
   iii. Lecture – DNA → Protein
   iv. Lecture – Restriction Enzymes

b. **LAB: PV92 Amplication via PCR**
   i. amplification of intronic region of human chromosome 16 from DNA isolated from buccal cells

c. **LAB: DNA Restriction Enzyme Simulations**
   i. using Microsoft Word, λ DNA is cut with 3 endonucleases to predict "banding pattern"
   ii. NEB Cutter

d. **LAB: DNA Restriction Analysis**
   i. predict the outcome of the restriction enzymes *Bam*HI, *Eco*RI, and *Hind*III on λ DNA
   ii. stain with SYBR-Safe Gel Stain
   iii. construct standard curve using 1kbp and 500bp DNA Ladders from BIO-RAD
   iv. construct standard curve using Logger Pro

e. **LAB: Effects of DNA Methylation on DNA Restriction**
   i. compare and contrast restriction with and without methylation

f. Biotechnology
   i. Lecture – Bacterial Genetics
   ii. Lecture – Genetic Engineering/Constructing a Plasmid
   iii. Activity – Plasmid Mapping

g. **LAB: Bacterial Culture Techniques**
   i. proper sterile technique to prepare bacterial media with an autoclave
   ii. preparation of LB and LB/amp plates
   iii. streaking practice of *E. coli* cultures onto LB and LB/amp agar plates
   iv. preparation of small-scale suspension of *E.coli* in stationary phase
   v. plating of bacteria onto media plates
   vi. preparation of a mid-log culture of *E.coli*

h. **LAB: Rapid Transformation of *E.coli* with Plasmid DNA**
   i. *E. coli* MM294 transformed with pAMP, pGREEN, and pVIB
   ii. heat shock time and concentration of plasmid variants studied

i. **LAB: Assay for an Antibiotic Resistance Enzyme**
   i. isolation of β-lactamase from MM294/pAMP cells
   ii. qualitative vs. quantitative analysis of β-lactamase assay

j. **LAB: Purification and Identification of Recombinant GFP**
   i. purification of GFP from MM294/pGREEN cells via HIC resin
   ii. SDS-PAGE analysis of purified GFP

k. **LAB: Purification and Identification of Plasmid DNA**
   i. plasmid miniprep of pAMP from MM294/pAMP
   ii. restriction analysis of purified plasmids

END-OF-YEAR PROJECTS

V. Genetic Engineering 101

a. **LAB: Recombination of Antibiotic Resistance Genes**
   i. using *E.coli* MM294, pAMP, pKAN, *Bam*HI, *Hin*dIII, and t4 ligase, a new plasmid will be constructed containing both ampicillin and kanamycin resistant genes

b. **LAB: Transformation of *E. coli* with Recombinant Plasmid DNA**
   i. transform *E. coli* MM294 with engineered plasmids
   ii. calculate transformation efficiencies
   iii. compare with miniprep DNA

c. **LAB: Purification and Identification of Plasmid DNA**
   i. plasmid miniprep of engineered plasmid
   ii. restriction analysis of purified plasmid

VI. Building a Gene Library of λ DNA

a. **LAB: Recombination of Antibiotic Resistance Genes**
   i. using *E.coli* MM294, pBLU, λ DNA, *Bam*HI, *Hin*dIII, and t4 ligase, a new plasmid will be constructed containing fragments of λ DNA
   ii. through the steps of the previous project, colonies of engineered cells containing segments of λ DNA will be cultured
   iii. "library" cells will have their contents verified via miniprep and restriction digest

VII. Fluorescent *E. coli* Art Show!

a. **LAB: 'Nuff Said! This is the super fun stuff!**