

# BIOCHEMISTRY/MOLECULAR BIOLOGY @ CCHS 2019-2020

## 1<sup>st</sup> 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 → Quaternary
- 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

## 2<sup>nd</sup> 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 Amplification 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,  $\lambda$  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  $\lambda$  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  $\beta$ -lactamase from MM294/pAMP cells
  - ii. qualitative vs. quantitative analysis of  $\beta$ -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, *Hind*III, 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 DNA0000**
  - i. plasmid miniprep of engineered plasmid
  - ii. restriction analysis of purified plasmid

### VI. Building a Gene Library of $\lambda$ DNA

- a. **LAB: Recombination of Antibiotic Resistance Genes**
  - i. using *E.coli* MM294, pBLU,  $\lambda$  DNA, *Bam*HI, *Hind*III, and t4 ligase, a new plasmid will be constructed containing fragments of  $\lambda$  DNA
  - ii. through the steps of the previous project, colonies of engineered cells containing segments of  $\lambda$  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!**