CHEMISTRY 567

OVERALL BIOCHEMISTRY LABORATORY COURSE SCHEDULE – SPRING '20

Week	Monday 1:00 pm lecture	Tuesday, 2:00 pm Lab	Thursday, 2:00 pm Lab
1 Jan 20	Holiday		1. Course Overview 2. Biochemistry Boot Camp- all the things you have already learned but may have forgotten. 3. obtain key cards for access to Chemistry computer room.
2 Jan 27	Introductory Lecture on Photosynthetic Reaction Center Experiment	1. List to obtain key cards for access to Chemistry computer room. 2. Biochemistry Boot Camp- all the things you have already learned but may have forgotten. (continued) 3. Lecture on Lab Techniques part 1	1. Lecture on Lab Techniques part 2, 2. Locker Check-in 3. Interactive Biochemistry Computer Techniques (Chem computer room, GMCS234). All students must go through the computer-based interactive section of the Photosynthetic Reaction Center experiment **prior** to the lab phase beginning on Feb 5
3 Feb 3	Introductory Lecture on Lactate Dehydrogenase Kinetics Experiment	1. Begin Photosystems Experiment - isolation of spinach thylakoids **(Introduction and Flow Chart due at beginning of class.)**	1. Finish Photosystems Experiment- separation and characterization of the photosystems
4 Feb 10	**EXAM on Photosynthetic Reaction Center Experiment (including lab techniques lecture)**	1. Begin Lactate Dehydrogenase Kinetics Experiment **(Introduction and Flow Chart due at beginning of class.)** a) Progress Curves; initial velocities. b) Effects of enzyme concentration on initial velocity.	**1. Lab Report on Photosystems Experiment due at beginning of class.** 2. Continue Lactate Dehydrogenase Kinetics pH effects on velocity.
5	First Introductory Lecture on Aldolase	1. Continue Lactate Dehydrogenase Kinetics-	1. Begin Purification and Characterization of

Feb 17	Experiment	Velocity vs. substrate concentration plot. Inhibition kinetics with cibacron blue.	Aldolase experiment **(Introduction and Flow Chart for first four days of Aldolase Experiment due at beginning of class, i.e., purification, dialysis, phosphocellulose column chromatography, gel filtration chromatography, etc.)** a)Isolation of chicken breast muscle cytosol b) Prepare gel filtration column. c) Salt in excess sample
6 Feb 24	**EXAM on Lactate Dehydrogenase Kinetics Experiment.**	1. **LAB REPORT DUE at beginning of class on LDH Kinetics.** 2. Continue Aldolase experiment a) Ammonium sulfate precipitation of aldolase b) Determine void volume of Sephadex gel filtration column c) Prepare phosphocellulose ion exchange/affinity column d) Overnight dialysis	1. Continue Aldolase experiment a) Run phosphocellulose column. b) Determine elution volume of first set of gel filtration column standards. c) Start protein concentration and specific activity assays.
7 March 2	Second Introductory Lecture on Aldolase Experiment.	1. Continue Aldolase experiment a) Run aldolase on gel filtration column. b) Continue protein concentration and specific activity assays. c) Prepare SDS polyacrylamide gel.	**1. Introduction and Flow Chart for second part of Aldolase Experiment (SDS gel and immunoblot) due.** 2. Continue Aldolase experiment a) Run SDS PAGE gel and blot to membrane (western transfer) b) Determine elution volume of second set of gel filtration column standards
	(Continued)	(Continued)	(Continued)

	Monday 1:00 pm lecture	Tuesday, 2:00 pm Lab	Thursday, 2:00 pm Lab
8 Mar 9	Introductory Lecture on Plasmid Prep	1. Continue Aldolase experiment a) Develop Immunoblot. b) Finish elution volume determination of final gel filtration column standards.	Kathy guest lectures Forensic Anthropology
9 Mar 16	**Continue** introductory lecture on plasmid prep	1. Begin Plasmid Preparation Experiment a) Prepare and autoclave media for 1 liter cultures b) Start overnight 25 ml culture from glycerol stock.	1. **LAB REPORT on Aldolase Experiment DUE at beginning of Class (2:00 PM).** 2. Continue Plasmid Prep a) At **1:00 pm**, innoculate 1 liter cultures b) 2:00 pm: Begin monitoring cell growth by periodic readings of A_{600} c) Add chloramphenicol. d) Incubate overnight. TA will spin down the cells the following morning. You may observe or help if you would like to.
10 Mar 23	EXAM on Aldolase Module	**1. Introduction and Flow Chart for Plasmid Prep due at beginning of class** 2. Continue Plasmid Prep a) Prepare clear lysate b) Set up first CsCl gradient c) Start first equilibrium gradient centrifugation run	1. Introductory Lecture on the Polymerase Chain Reaction (PCR) experiment, synthetic DNA oligonucleotide synthesis, and gel electrophoresis of nucleic acids (Stumph) 2. Continue Plasmid Prep a) Take down first CsCl gradient b) Start second CsCl gradient centrifugation
	SPRING BREAK		
11 Apr 6	Continue Introductory Lecture on PCR/STR's.	**1**. Start PCR/STR experiment **(Introduction and Flow Chart Due at beginning of class)** 2. Isolate cheek cell DNA 3. Continue Plasmid Prep- Take down second CsCl gradient, extract ethidium bromide, and precipitate the DNA	1. Continue PCR/STR experiment- Set up PCR a) Pour agarose / acrylamide gels 2. Continue Plasmid Prep a) Spin down precipitated DNA, dissolve in water, and reprecipitate with ethanol. b) Spin down reprecipitated DNA, air dry pellet, resuspend in 1 ml TE buffer
12 Apr 13	Getting a job/resumes	1. Run gels with DNA samples 2. Finish Plasmid Prep a) Take absorbance readings of plasmid preps; calculate yield 3. Introductory Lecture on DNA Sequencing	1. Read STR gel 2. Video on DNA sequencing 3. Practice reading sequencing gel autoradiograms

13 Apr 20	Lecture- Real time PCR	**1. LAB REPORT on Plasmid Prep DUE at beginning of class.** 2. Tissue culture and stem cells lecture	Introductory Lecture on GST-SH2 Expression and Purification.
14 Apr 27	Next generation sequencing	1. Begin GST-SH2 Expression and Purification Experiment. **(Introduction and Flow Chart for GST-SH2 Expression and Purification Due at beginning of class)** 2. Lyse bacterial cells and bind supernatant to resin. 3. Elute protein from resin.	1. **Homework on DNA Sequencing DUE at beginning of class.** 2. **Lab Report on PCR/STR Due at beginning of class** 3. Continue GST-SH2 Expression and Purification Run protein samples on gel, take down, stain gel
15 May 4	**Exam on Recombinant DNA module, including GST-SH2.**	1. Finish GST-SH2 Expression and Purification, destain gel, and photograph.. 2. Check out.	**Lab Report on GST-SH2 due - 11:00 pm. (email) May 11**
			No final exam