

CHEMISTRY 567

OVERALL BIOCHEMISTRY LABORATORY COURSE SCHEDULE – FALL '15

Week	Monday 2:00 pm lecture	Tuesday, 2:00 pm Lab	Thursday, 2:00 pm Lab
<u>1</u> Aug 24	Course Overview Biochemistry Boot Camp	Boot camp continued... Lecture on lab techniques, part 1 obtain key cards for access to Chemistry computer room.	1. 2:00 pm: Lecture on lab techniques, part 2 2. 3:30 pm: Interactive Biochemistry Computer Techniques (Chem computer room, GMCS245). All students must go through the computer-based interactive section of the Photosynthetic Reaction Center experiment prior to the lab phase beginning on Sept 3
<u>2</u> Aug 31	Introductory Lecture on Photosynthetic Reaction Center Experiment	1. Introductory Lecture on Lactate Dehydrogenase Kinetics Experiment 2. Locker Check-in	1. Begin Photosystems Experiment - isolation of spinach thylakoids (Introduction and Flow Chart due at beginning of class.)
<u>3</u> Sept 7	<u>Holiday</u>	1. Finish Photosystems Experiment- separation and characterization of the photosystems	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.
<u>4</u> Sept 14	EXAM on Photosynthetic Reaction Center Experiment (including lab techniques lecture)	1. Lab Report on Photosystems Experiment due at beginning of class. 2. Continue Lactate Dehydrogenase Kinetics pH effects on velocity.	1. Continue Lactate Dehydrogenase Kinetics- Velocity vs. substrate concentration plot. Inhibition kinetics with cibacron blue.
<u>5</u> Sept 21	First Introductory Lecture on Aldolase Experiment	Kathy guest lectures in Forensic Anthropology, no class today	1. Begin Purification and Characterization of 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 <u>excess</u> sample
<u>6</u> Sept 28	EXAM on Lactate Dehydrogenase Kinetics Experiment.	1. LAB REPORT DUE at beginning of class on LDH Kinetics. 2. Continue Aldolase experiment	1. Continue Aldolase experiment a) Run phosphocellulose column. b) Determine elution volume of first set of gel

		<ul style="list-style-type: none"> 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 	<ul style="list-style-type: none"> filtration column standards. c) Start protein concentration and specific activity assays.
<u>7</u> Oct 5	Second Introductory Lecture on Aldolase Experiment.	<ul style="list-style-type: none"> 1. Continue Aldolase experiment <ul style="list-style-type: none"> a) Run aldolase on gel filtration column. b) Continue protein concentration and specific activity assays. c) Prepare SDS polyacrylamide gel. 	<ul style="list-style-type: none"> 1. Introduction and Flow Chart for second part of Aldolase Experiment (SDS gel and immunoblot) due. 2. Continue Aldolase experiment <ul style="list-style-type: none"> a) Run SDS PAGE gel and blot to membrane (western transfer) b) Determine elution volume of second set of gel filtration column standards
<u>8</u> Oct 12	Introductory Lecture on Plasmid Prep	<ul style="list-style-type: none"> 1. Continue Aldolase experiment <ul style="list-style-type: none"> a) Develop Immunoblot. b) Finish elution volume determination of final gel filtration column standards. 	Finish Aldolase (if needed, otherwise, day off)
	(Continued)	(Continued)	(Continued)

	Monday 1:00 pm lecture	Tuesday, 2:00 pm Lab	Thursday, 2:00 pm Lab
<u>9</u> Oct 19	Continue introductory lecture on plasmid prep	<ol style="list-style-type: none"> 1. Begin Plasmid Preparation Experiment <ol style="list-style-type: none"> a) Prepare and autoclave media for 1 liter cultures b) Start overnight 25 ml culture from glycerol stock. 	<ol style="list-style-type: none"> 1. Introduction and Flow Chart for Plasmid Prep due at beginning of class 2. Continue Plasmid Prep <ol style="list-style-type: none"> a) At 1:00 pm, inoculate 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.
<u>10</u> Oct 26	EXAM on Aldolase Module	<ol style="list-style-type: none"> 1. LAB REPORT on Aldolase Experiment DUE at beginning of Class (2:00 PM). 2. Continue Plasmid Prep <ol style="list-style-type: none"> a) Prepare clear lysate b) Set up first CsCl gradient c) Start first equilibrium gradient centrifugation run 	<ol style="list-style-type: none"> 1. Introductory Lecture on the Polymerase Chain Reaction (PCR) experiment, synthetic DNA oligonucleotide synthesis, and gel electrophoresis of nucleic acids 2. Continue Plasmid Prep <ol style="list-style-type: none"> a) Take down first CsCl gradient b) Start second CsCl gradient centrifugation
<u>11</u> Nov 2	Continue Introductory Lecture on PCR/STR's.	<ol style="list-style-type: none"> 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 	<ol style="list-style-type: none"> 1. Continue PCR/STR experiment- Set up PCR <ol style="list-style-type: none"> a) Pour agarose / acrylamide gels 2. Continue Plasmid Prep <ol style="list-style-type: none"> 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
<u>12</u> Nov 9	Getting a job/resumes	<ol style="list-style-type: none"> 1. Run gels with DNA samples 2. Finish Plasmid Prep <ol style="list-style-type: none"> a) Take absorbance readings of plasmid preps; calculate yield 3. Introductory Lecture on DNA Sequencing 	<ol style="list-style-type: none"> 1. Read STR gel 2. Video on DNA sequencing 3. Practice reading sequencing gel autoradiograms
<u>13</u> Nov 16	Lecture- Real time PCR	<ol style="list-style-type: none"> 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.

<u>14</u> Nov 23	No class	No class	HW: Large scale carbohydrate digestion experiment
<u>15</u> Nov 30	Next generation sequencing lecture	<ol style="list-style-type: none"> 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. 	<ol style="list-style-type: none"> 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
<u>16</u> Dec 7	Exam on Recombinant DNA module, including GST-SH2.	<ol style="list-style-type: none"> 1. Finish GST-SH2 Expression and Purification, destain gel, and photograph.. 2. Check out. 	No Class
<u>Dec 14</u>	Lab Report on GST-SH2 due - 11:00 pm. (email)		No final exam