

# CHEMISTRY 567

## OVERALL BIOCHEMISTRY LABORATORY COURSE SCHEDULE – FALL '19

Week	Monday 11:00 am lecture	Tuesday, 3:00 pm Lab	Thursday, 3:00 pm Lab
<u>1</u> Aug 26	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 <b>prior</b> to the lab phase beginning on Sept 5
<u>2</u> Sept 2	Labor day	1. Introductory Lecture on Photosynthetic Reaction Center Experiment 2. Locker Check-in	1. Begin Photosystems Experiment - isolation of spinach thylakoids ( <b>Introduction and Flow Chart due at beginning of class.</b> )
<u>3</u> Sept 9	1. Introductory Lecture on Lactate Dehydrogenase Kinetics Experiment	1. Finish Photosystems Experiment- separation and characterization of the photosystems	1. Begin Lactate Dehydrogenase Kinetics Experiment ( <b>Introduction and Flow Chart due at beginning of class.</b> ) a) Progress Curves; initial velocities. b) Effects of enzyme concentration on initial velocity.
<u>4</u> Sept 16	<b>EXAM on Photosynthetic Reaction Center Experiment (including lab techniques lecture)</b>	1. <b>Lab Report on Photosystems Experiment due at beginning of class.</b> 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 23	First Introductory Lecture on Aldolase Experiment	Kathy guest lectures in Forensic Anthropology, no class today	1. Begin Purification and Characterization of Aldolase experiment ( <b>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.</b> ) a) Isolation of chicken breast muscle cytosol b) Prepare gel filtration column. c) Salt in <u>excess</u> sample
<u>6</u> Sept 30	<b>EXAM on Lactate Dehydrogenase Kinetics Experiment.</b>	1. <b>LAB REPORT DUE at beginning of class on LDH Kinetics.</b> 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"> <li>a) Ammonium sulfate precipitation of aldolase</li> <li>b) Determine void volume of Sephadex gel filtration column</li> <li>c) Prepare phosphocellulose ion exchange/affinity column</li> <li>d) Overnight dialysis</li> </ul>	<ul style="list-style-type: none"> <li>filtration column standards.</li> <li>c) Start protein concentration and specific activity assays.</li> </ul>
<u>7</u> Oct 7	Second Introductory Lecture on Aldolase Experiment.	<ul style="list-style-type: none"> <li>1. Continue Aldolase experiment <ul style="list-style-type: none"> <li>a) Run aldolase on gel filtration column.</li> <li>b) Continue protein concentration and specific activity assays.</li> <li>c) Prepare SDS polyacrylamide gel.</li> </ul> </li> </ul>	<ul style="list-style-type: none"> <li>1. <b>Introduction and Flow Chart for second part of Aldolase Experiment (SDS gel and immunoblot) due.</b></li> <li>2. Continue Aldolase experiment <ul style="list-style-type: none"> <li>a) Run SDS PAGE gel and blot to membrane (western transfer)</li> <li>b) Determine elution volume of second set of gel filtration column standards</li> </ul> </li> </ul>
<u>8</u> Oct 14	<b>Introductory Lecture on Plasmid Prep</b>	<ul style="list-style-type: none"> <li>1. Continue Aldolase experiment <ul style="list-style-type: none"> <li>a) Develop Immunoblot.</li> <li>b) Finish elution volume determination of final gel filtration column standards.</li> </ul> </li> </ul>	Finish Aldolase (if needed, otherwise, day off)
	(Continued)	(Continued)	(Continued)

	Monday 11:00 am lecture	Tuesday, 3:00 pm Lab	Thursday, 3:00 pm Lab
<u>9</u> Oct 21	<b>Continue</b> introductory lecture on plasmid prep	<ol style="list-style-type: none"> <li>1. Begin Plasmid Preparation Experiment               <ol style="list-style-type: none"> <li>a) Prepare and autoclave media for 1 liter cultures</li> <li>b) Start overnight 25 ml culture from glycerol stock.</li> </ol> </li> </ol>	<ol style="list-style-type: none"> <li>1. <b>Introduction and Flow Chart for Plasmid Prep due at beginning of class</b></li> <li>2. Continue Plasmid Prep               <ol style="list-style-type: none"> <li>a) At <b>1:30 pm</b>, inoculate 1 liter cultures</li> <li>b) 3:00 pm: Begin monitoring cell growth by periodic readings of <math>A_{600}</math></li> <li>c) Add chloramphenicol.</li> <li>d) Incubate overnight. TA will spin down the cells the following morning. You may observe or help if you would like to.</li> </ol> </li> </ol>
<u>10</u> Oct 28	<b>EXAM on Aldolase Module</b>	<ol style="list-style-type: none"> <li>1. <b>LAB REPORT on Aldolase Experiment DUE at beginning of Class (2:00 PM).</b></li> <li>2. Continue Plasmid Prep               <ol style="list-style-type: none"> <li>a) Prepare clear lysate</li> <li>b) Set up first CsCl gradient</li> <li>c) Start first equilibrium gradient centrifugation run</li> </ol> </li> </ol>	<ol style="list-style-type: none"> <li>1. Introductory Lecture on the Polymerase Chain Reaction (PCR) experiment, synthetic DNA oligonucleotide synthesis, and gel electrophoresis of nucleic acids</li> <li>2. Continue Plasmid Prep               <ol style="list-style-type: none"> <li>a) Take down first CsCl gradient</li> <li>b) Start second CsCl gradient centrifugation</li> </ol> </li> </ol>
<u>11</u> Nov 4	<b>Continue Introductory Lecture on PCR/STR's.</b>	<ol style="list-style-type: none"> <li>1. Start PCR/STR experiment (<b>Introduction and Flow Chart Due at beginning of class</b>)</li> <li>2. Isolate cheek cell DNA</li> <li>3. Continue Plasmid Prep- Take down second CsCl gradient, extract ethidium bromide, and precipitate the DNA</li> </ol>	<ol style="list-style-type: none"> <li>1. Continue PCR/STR experiment- Set up PCR               <ol style="list-style-type: none"> <li>a) Pour agarose / acrylamide gels</li> </ol> </li> <li>2. Continue Plasmid Prep               <ol style="list-style-type: none"> <li>a) Spin down precipitated DNA, dissolve in water, and reprecipitate with ethanol.</li> <li>b) Spin down reprecipitated DNA, air dry pellet, resuspend in 1 ml TE buffer</li> </ol> </li> </ol>
<u>12</u> Nov 11		<ol style="list-style-type: none"> <li>1. Run gels with DNA samples</li> <li>2. Finish Plasmid Prep               <ol style="list-style-type: none"> <li>a) Take absorbance readings of plasmid preps; calculate yield</li> </ol> </li> <li>3. Introductory Lecture on DNA Sequencing</li> </ol>	<ol style="list-style-type: none"> <li>1. Read STR gel</li> <li>2. Video on DNA sequencing</li> <li>3. Practice reading sequencing gel autoradiograms</li> </ol>
<u>13</u> Nov 18	Lecture- Real time PCR	<ol style="list-style-type: none"> <li>1. <b>LAB REPORT on Plasmid Prep DUE at beginning of class.</b></li> <li>2. Tissue culture and stem cells lecture</li> </ol>	Introductory Lecture on GST-SH2 Expression and Purification.

<u>14</u> Nov 25	Getting a job/resumes		HW: Large scale carbohydrate digestion experiment
<u>15</u> Dec 2	Next generation sequencing lecture	<ol style="list-style-type: none"> <li>1. Begin GST-SH2 Expression and Purification Experiment. (<b>Introduction and Flow Chart for GST-SH2 Expression and Purification Due at beginning of class</b>)</li> <li>2. Lyse bacterial cells and bind supernatant to resin.</li> <li>3. Elute protein from resin.</li> </ol>	<ol style="list-style-type: none"> <li>1. <b>Homework on DNA Sequencing DUE at beginning of class.</b></li> <li>2. <b>Lab Report on PCR/STR Due at beginning of class</b></li> <li>3. Continue GST-SH2 Expression and Purification Run protein samples on gel, take down, stain gel</li> </ol>
<u>16</u> Dec 9	<b>Exam on Recombinant DNA module, including GST-SH2.</b>	<ol style="list-style-type: none"> <li>1. Finish GST-SH2 Expression and Purification, destain gel, and photograph.</li> <li>2. Check out.</li> </ol>	No Class
<u>Dec 16</u>	<b>Lab Report on GST-SH2 due - 11:00 pm. (email)</b>		No final exam