

## Syllabus

### 03-392: Mini Microbiology Laboratory

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	<b>Mondays, 1:30 - 4:20 pm</b>	<b>Wednesdays, 1:30 - 4:20pm</b>
<b>Week #1</b>	<p><u>Room:</u> DH-2303(1:30-3pm)  <u>Lecturer:</u> Borysenko  <u>Purposes:</u> <b>Microbe Introduction</b></p> <ol style="list-style-type: none"> <li>1) Introduction/Purpose Statement.</li> <li>2) Biosafety: clean area with ethanol, picking colonies, transferring loop/ flames, agar plate streaking, how to use autoclave, WASH HANDS.</li> <li>3) General shapes (rod, sphere, cork-screw, single cells, flagella, chains, pairs, clumps)</li> <li>4) Brownian motion: microbe or dust?</li> </ol> <p><u>Room:</u> DH-2302 (3-4:20 pm)  <u>Lecturer:</u> Andrew Lawson  <u>Purpose:</u></p> <ol style="list-style-type: none"> <li>1) <b>University Biosafety Lecture</b></li> </ol>	<p><u>Room:</u> Baker Hall 140F (1:30-3:20pm)  <u>Lecturer:</u> Borysenko  <u>Purposes:</u> <b>Mathematical Modeling/Spectroscopy</b></p> <ol style="list-style-type: none"> <li>1) Practice Built-in Mathematica 5.2 Functions: Statistics NonlinearFit, Nonlinear-Regress, ListPlot</li> <li>2) Solve coupled time-dependent equations</li> <li>3) ATCC website for K12 E. coli strain JM101</li> <li>4) Interpret Genotype entry on ATCC sheet.</li> </ol> <p><u>Room:</u> DH-2303 (3:30-4:20 pm)  <u>Lecturer:</u> Borysenko  <u>Purposes:</u></p> <ol style="list-style-type: none"> <li>1) Use Cary 50 UV/Vis spectrophotometer for absorbance and light scattering.</li> <li>2) Generate Standard Curve with Blue Dye #1: <u>Inductive Logic</u></li> <li>3) Determine unknown : <u>Deductive logic</u></li> </ol>
<b>Week #2</b>	<p><u>Room:</u> DH-2303-A Dark Room (1:30-3:30pm)  <u>Lecturer:</u> Jason Shields (Olympus Field Scientist)  <u>Purposes:</u> <b>Microscope Demo</b></p> <ol style="list-style-type: none"> <li>1) BX60 Olympus Demonstration</li> </ol> <p><u>Room:</u> DH-2303-A Dark Room (3:30-4:20 pm)  <u>Lecturer:</u> CW Borysenko  <u>Purposes:</u> <b>Flow Cytometer Demo</b></p> <ol style="list-style-type: none"> <li>1) Introduction to 4-channel Flow Cytometer: total cell count, fluorescence, compensation, histograms, 2D plots.</li> <li>2) Practice with JM101 that express GFP (use channel #1)</li> </ol>	<p><u>Room:</u> Baker Hall 140F Computer Cluster (1:30-3:30pm)  <u>Lecturer:</u> Borysenko  <u>Purposes:</u> <b>Fit/assess data using Mathematica</b></p> <ol style="list-style-type: none"> <li>1) Derive Growth Curves with Mathematica 5.2</li> <li>2) Use Data for yeast growth from: Gause GF (1969), Fig 8. The Struggle for Existence, Hafner, NY, NY.</li> <li>3) Stationary Phase due to: nutrient depletion, competition for space, contamination, initial density, etc. Include in models.</li> <li>4) Logistics equation: Apparent doubling time, apparent carrying capacity</li> </ol> <p><u>Room:</u> DH-2303 (3:30-4:20pm)  <u>Lecturer:</u> Borysenko  <u>Purposes:</u></p> <ol style="list-style-type: none"> <li>1) Inoculate O/N culture of JM101-GFP for Monday (I will treat with IPTC to express GFP on Sunday). <b>Autoclave</b> (in basement) 8-250 mL flasks.</li> </ol>

<p><b>Week #3</b></p>	<p><u>Room:</u> DH-2303A Dark Room (1:30-4:20pm)  <u>Lecturer:</u> Borysenko  <u>Purposes:</u> <b>Viability Determination by FC &amp; Serial Dilution Plating</b>  <b>1)</b> Viability measured by Flow Cytometry  <b>2)</b> Treat JM101 +/- GFP with viability stain propidium iodide (PI)  <b>3)</b> Analyze with Flow Cytometer and compensate if necessary.  <b>4)</b> Express data as histograms and 2D plots with GFP (chan#1) vs PI (chan#3).  <b>5)</b> Use same O/N culture, do 6-10x dilutions and put in incubator. Count cells on Wed.</p>	<p><u>Room:</u> DH-2303A Dark Room (1:30-4:20pm)  <u>Lecturer:</u> CW Borysenko  <u>Purposes:</u> <b>FC analysis of JM101 after freezing/OD660 light scattering for cell counting</b>  <b>1)</b> Penna TC, Chiarini E, Machoshvili IA, Ishii M, Pessoa A Jr. Intracellular release of recombinant green fluorescent protein(gfp(uv)) from E. coli. Appl Biochem Biotechnol. 2002 Spring; 98-100:791-802.  <b>2)</b> Repeat viability study from Monday as a function of # of freeze/thaw cycles  <b>3)</b> Analyze data as histograms and 2D graphs  <b>4)</b> Dilute JM101 culture in growth media (over about 2-log dilutions) and read light scattering in spec at 660nm. Graph data.</p>
<p><b>Week #4</b></p>	<p><u>Room:</u> DH-2303 Laboratory Room  <u>Lecturer:</u> Borysenko  <u>Purposes:</u> <b>Theory of cell mass vs per capita measurements</b>  <u>Summary of last week's experiments:</u>  <b>1)</b> OD 660nm light scattering  <b>2)</b> Klett values  <b>3)</b> Per capita values from flow cytometry  <b>4)</b> Viability by Serial Dilution Plating  <b>Laboratory Exercise:</b>  <b>4) Repeat:</b> Measure 5 dilutions of JM101 by each method above. Graph Results.  <b>5)</b> The literature value for particles with E coli shape/density is: 800 million/mL = 1.0 OD660 (Review physics of light scattering)</p>	<p><u>Room:</u> DH-2303 Laboratory Room  <u>Lecturer:</u> Borysenko  <u>Purposes:</u> <b>Collect Growth Data for JM101 as a function of Initial Densities / Fit to Mathematica Models.</b>  <b>1)</b> Inoculate 25 mL M9+ media with JM101 by loop from agar plate. Use 3 densities.  <b>2)</b> Assay cell numbers using any method from Monday every 45 min.  <b>3)</b> Fit data to logistics equations. Make conclusions about apparent rate constants and carrying capacities as a function of initial density.</p>
<p><b>Week #5</b></p>	<p><u>Room:</u> Baker Hall 140F Computer Cluster  <u>Lecturer:</u> Borysenko  <u>Purposes:</u> <b>Finish Fitting Lab Data from Wednesday to Mathematica 5.2 Equations</b>  <b>1)</b> Review Using Built-in functions for Mathematica  <b>2)</b> Review Using "Fitting Add-Ons": Statistics NonlinearFit, Nonlinear-Regress, ListPlot to assess goodness of fit.  <b>3)</b> Preview of Metabologica Control Theory</p>	<p><u>Room:</u> DH-2303 Lab Room  <u>Lecturer:</u> Borysenko  <u>Purposes:</u> <b>Explore JM101 Genotype and pGFPuv Vector: How Experimental conditions are Determined</b>  <b>1)</b> Go to ATCC website and obtain info for K12 E. coli JM101  <b>2)</b> Explain why M9+ complete media contain Vitamin B1, etc.  <b>3)</b> Why is JM101 considered NOT pathogenic?  <b>4)</b> Blast: copy a 20-mer sequence of the pGFPuv vector (from handout) and do a "blast" in the PubMed website. What is/are the GenBank Accession number(s) that match?  <b>5)</b> Why are Amp and IPTG included in the growth media when GFP is expressed?  <b>6)</b> Why is a beta-galactosidase-GPF chimera protein</p>

		produced in the transformed JM101 cells? 7) What does MCS stand for?
<b>Week #6</b>	<u>Room:</u> DH-2303 Laboratory Room <u>Lecturer:</u> Borysenko <b><u>Purposes:</u> Biology of Microbe stains</b> <b>1)</b> Christian Gram stains: counter stains <b>2)</b> Others: viability staining, DNA staining, plasmid staining, flagella staining	<u>Room:</u> DH-2303 Laboratory Room <u>Lecturer:</u> Borysenko <b><u>Purposes:</u> Microbe Morphology and Gram Staining</b> <b>1)</b> Perform Gram staining on: B. cereus, E. coli, Staph. epidermidis, Spirillum serpens. <b>2)</b> View (100x oil) with BX60 (See Week 2). <b>3)</b> Record staining results <b>4)</b> Classify by shape (see lecture #1)
<b>Week #7</b>	<u>Room:</u> DH-2303 Laboratory Room <u>Lecturer:</u> Prof. M Domach <b><u>Purposes:</u> Metabolic Control Theory and Computer Program Metabologica (developed by M Domach)</b> <b>1)</b> <u>Predictions</u> by Metabologica for JM101 wt and PB25 (JM101 with pyruvate kinase KO): a) Same doubling time b) Same carrying capacities c) PB25 can do this by "wasting" C-sources and producing more acetic acid in conditioned media, for example.	<u>Room:</u> DH-2303 Laboratory Room <u>Lecturer:</u> Borysenko <b><u>Purposes:</u> Challenge Metabologica Predictions with experimental measurements: Growth and Conditioned Media</b> <b>1)</b> Inoculate M9+ media from O/N growth with JM101 and another flask with PB25. Dilute so initial OD660 = 0.10 <b>2)</b> Collect Growth Curves and fit to Logistics equation <b>3)</b> Filter conditioned media and assay for acetic acid by HPLC (%95 water/%5 acetonitrile, 1 mL/min, detector = 405 nm, Discover C18 reverse-phase column). <b>4)</b> Compare results to Metabologica Predictions from Monday