NUTR 0225: Introduction to Modern Biology Techniques
Fall 2018

Class Meetings: Tuesday, 1:30-3:00, 711 Washington Street, HNRCA mezzanine conference room
Students will check in at the security desk of the Human Nutrition Research Center on Aging and will be escorted from the first-floor lounge area to the classroom. It is vital that foreign students have completed the ARS230 form and received approval.

Instructor(s): Martin Obin, PhD, martin.obin@tufts.edu; 617 272 6607

Instructor Office Hours: Tuesdays 12:00-1:15, HNRCA Mezzanine Conference Room or by appointment

Teaching Asst.: N/A

Teaching Asst. Office Hours: N/A

Semester Hour Units: 1.5 SHUs

Prerequisites: Graduate standing, undergraduate biochemistry or instructor permission

Course Description: This intensive 12-week course will familiarize you with techniques employed in nutrition science research at the molecular, cell, tissue, whole organism and population levels. An understanding of these techniques and their application is essential for reading the nutrition science literature, developing skills as a researcher and appreciating the goals, current limitations and future potential of modern nutrition science research. Eight (at times overlapping) classifications of techniques and experimental approaches will be presented over 12 weeks:

1. Partitioning techniques for biomolecule separation, and downstream analysis by Mass Spectrometry
2. Physical separation of cell and tissue constituents
3. Analysis of cell constituents and biomolecules using antibody-based techniques in conjunction with...
4. Fluorescence, Bioluminescence and Imaging techniques.
5. Gene Expression: Analysis and Manipulation
6. ‘Omics’ and Systems Biology
7. Data Science (Informatics, Computational Biology, ‘Virtual’ Communities/Consortia))
8. Bioengineering and Synthetic Biology

For each technique we will emphasize the underlying principles/theory, work-flow and data output. An understanding of the strengths, limitations and combinatorial implementation of these techniques will facilitate your development as an experimentalist and scholar. An additional goal of this course is to familiarize you with the “virtual” (i.e., online) communities of scientists and the vast resources available to you in public domain websites managed by scientific and information consortia.

Course Objectives:

- To develop basic understanding of the theory, technology, methodology and use of techniques and experimental approaches in modern nutrition research.
- To develop student appreciation of how these different experimental techniques/approaches can be employed in a complementary and mutually-enforcing manner to address a nutrition research question in depth.
- By so doing, to develop student’s ability to read the nutrition science literature and creatively design, conduct and interpret nutrition science experiments during their graduate careers and beyond.
• To provide historical perspective to- and an understanding of- different modes of scientific inquiry and how seemingly incompatible approaches (e.g., reductionism vs systems biology, hypothesis-driven vs hypothesis-independent, model testing vs model improvement) can be used in concert to obtain increased experimental power and insight.

• By exposing students to the diversity of research techniques and fields of study, facilitate the development of Practicum (NUTR 236) projects, graduate research and career trajectories.

**Texts or Materials:** The course material is predominantly web-based, with emphasis on demonstration and interactive videos and supporting lectures. Additional materials, including journal articles are also incorporated to demonstrate “real-world” applicability. Several units have assignments beyond the reading that must be turned in at the beginning of class. In addition, a weekly “Study Guide for The Perplexed” that outlines specific learning objectives for each class will be available by the Friday preceding each class. All course materials will be available on Canvas: https://canvas.tufts.edu.

**Academic Conduct:** Each student is responsible for upholding the highest standards of academic integrity, as specified in the Friedman School’s Policies and Procedures Handbook and Tufts University policies (http://students.tufts.edu/student-affairs/student-life-policies/academic-integrity-policy). It is the responsibility of each student to understand and comply with these standards, as violations will be sanctioned by penalties ranging from failure on an assignment and the course to dismissal from the school.

**Classroom Conduct:** Students are expected to prepare for each class and actively participate in class discussion (40% of grade). If you are confused by or have questions about the assigned material, please come to office hours (12:00-1:15 Tuesday or by appointment) before the class to have questions addressed.

**Assessment and Grading:** This course is graded S/U. A “Satisfactory” grade requires an overall average of 80% (B-) or above. Each class (including the first class) will begin with a 15-20-minute quiz, followed by a discussion of the answers to the quiz and expanded discussion of techniques covered in your reading. The quiz will require short answers (word, phrase or several cogent sentences) and increasingly throughout the 12-week course, diagrams/cartoons depicting experimental design, work-flow and data output. Grading is based on aggregate of the quizzes (60%) and classroom participation (40%) (see below).

**Classroom Participation:** Classroom participation for each class is graded numerically as Excellent (95%), Satisfactory (85%) or Unsatisfactory (65%). Students who actively participate in class discussion and demonstrate a good understanding of the assigned material will receive an Excellent (95%) grade for that class. Students who participate in class discussion but demonstrate inadequate comprehension of the material will receive a Satisfactory (85%) grade for that class. Students who either fail to participate when questions are posed to the entire class and/ or who demonstrate lack of familiarity with the assigned material when directly called upon will receive an Unsatisfactory grade (65%).

Participating in classroom discussions can be difficult for some students, particularly those for whom English is a second language. If you are such a student, every effort will be made to accommodate you. If you tend to ‘freeze up’ when called upon in class, you should come to class prepared to ask questions about the assigned material. Please remember, there is no such thing as a “dumb question.” If you are confused by some aspect of the assigned reading or the instructor’s presentation, it is almost a certainty that someone (perhaps everyone!) else in the class is similarly confused. The classroom participation grade is based on the frequency and quality of your contributions to post-quiz discussions.

**Final Grade Criteria:** Grading (S/U) for the course will be calculated as follows:

- 12 in-class exams (5% each) 60% of final grade
- 8 highest classroom participation grades (5% each) 40% of final grade
**Instructions for Submission of Assignments and Exams:** There is a two-part assignment requiring on-line literature navigation that is to be turned in at the beginning of the first class (September 4th). For assignments that are turned in late, 10 points will be deducted for every day that the assignment is late. Students are expected to attend class and take quizzes on the specified dates and times unless otherwise arranged ahead of time with the course instructor. Last-minute absences from exams due to illness will require a note from the doctor in order to re-take the exam at a later date.

**Accommodation of Disabilities:** Tufts University is committed to providing equal access and support to all students through the provision of reasonable accommodations so that each student may access their curricula and achieve their personal and academic potential. If you have a disability that requires reasonable accommodations please contact the Friedman School Assistant Dean of Student Affairs at 617-636-6719 to make arrangements for determination of appropriate accommodations. Please be aware that accommodations cannot be enacted retroactively, making timeliness a critical aspect for their provision.

**Tufts WebEx:** Friedman’s on-campus courses may be offered by Tufts WebEx ([https://it.tufts.edu/webex](https://it.tufts.edu/webex)) on days when the Boston campus is closed due to weather or a temporary cancellation issue. Students should expect to be notified by email in the event that class is cancelled and will be provided with the WebEx link for students to use for any remote class sessions. Also, any relevant course slides or materials will be made available on Canvas. The WebEx will be recorded and posted on Canvas when completed. If an on-campus Examination/Presentation was scheduled on a day when the Boston campus is closed due to weather or a temporary cancellation issue, the exam/presentation will be rescheduled for an alternate on-campus class session date.

**Diversity Statement:** We believe that the diversity of student experiences and perspectives is essential to the deepening of knowledge in this course. We consider it part of our responsibility as instructors to address the learning needs of all of the students in this course. We will present materials that are respectful of diversity: race, color, ethnicity, gender, age, disability, religious beliefs, political preference, sexual orientation, gender identity, socioeconomic status, citizenship, language, or national origin among other personal characteristics.

**Course Topics and Assignment Schedule at a Glance:** This schedule is subject to modification at the instructor’s discretion.

<table>
<thead>
<tr>
<th>DATE OF CLASS</th>
<th>COURSE TOPIC</th>
<th>LECTURER</th>
<th>ASSIGNMENTS DUE</th>
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<td>September 4</td>
<td>Scientific Inquiry, Info Tools</td>
<td>Obin</td>
<td>Literature download; article summary; quiz</td>
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<tr>
<td>September 11</td>
<td>Partitioning Techniques</td>
<td>Obin</td>
<td>quiz</td>
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<td>September 18</td>
<td>Mass Spectrometry</td>
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<td>quiz</td>
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<td>September 25</td>
<td>Cell Culture/Fractionation</td>
<td>Obin</td>
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<td>October 2</td>
<td>Electrophoresis</td>
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<tr>
<td>October 9</td>
<td>NO CLASS/MONDAY SCHEDULE</td>
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<td>October 16</td>
<td>Antibodies, Fluorescence</td>
<td>Obin</td>
<td>quiz</td>
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<td>October 23</td>
<td>Imaging Techniques</td>
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<td>October 30</td>
<td>Measuring Gene Expression</td>
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<td>November 6</td>
<td>Manipulating Gene Expression I</td>
<td>Obin</td>
<td>quiz</td>
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Detailed Description of Course Topics, Assignment Schedule, and the Learning Objectives for Each Class Session:

September 4: Levels of Biological Organization; Modes of Scientific Inquiry; Information Tools

Learning Objectives:
1. List the hierarchical levels of biological organization and suggest a nutrition research question at each level.

2. Be able to define and provide an example of: hypothesis, theory, law, paradigm, deduction, induction

3. List the key steps in hypothesis-driven research and explain why the method is often referred to as the “hypothetico-deductive method”

4. Define and contrast “reductionism” and “systems biology” and describe how the two approaches can be complementary.

5. Be able to define and provide an example of “mechanism-based” and “mechanism-free” research.

6. Demonstrate the ability to navigate the Tufts Hirsh Health Sciences Library to access specified journal articles and databases.

Required Reading/Assignment

A. Levels of Biological Organization;
   https://biologywise.com/levels-of-biological-organization (review)

B. Modes and Methodology of Scientific Inquiry
   1. Scientific Method(s)
      https://explorable.com/what-is-a-paradigm
      https://www.livescience.com/20896-science-scientific-method.html (also understand “hypothesis”, “theory”, “law”, deductive vs inductive reasoning)
      https://www.youtube.com/watch?v=uWuNfhDvZz8 (3 min, 36s)
      http://www.zmescience.com/science/scientific-method-steps/
      https://www.thoughtco.com/elements-of-a-good-hypothesis-609096
      https://www.thoughtco.com/null-hypothesis-examples-609097
      https://www.youtube.com/watch?v=-X8XfOjdTQ (8 min, 56 s)

   2. Reductionist vs Systems Biology Approaches
      https://explorable.com/scientific-reductionism
      http://www.science20.com/knocking_lignocellulosic_biomass/reductionism_and_systems_thinking_complementary_scientific_lenses

   3. Mechanistic vs Mechanism-Free Science
      Casadevall and Fang, 2009, Mechanistic Science (available in Files in folder “week 1 additional readings”)
      https://www.wired.com/2008/06/pb-theory/
C. Information Tools and On-line Knowledge Communities

https://hirshlibrary.tufts.edu/
https://hirshlibrary.tufts.edu/find/databases
https://hirshlibrary.tufts.edu/find/ejournals
http://researchguides.library.tufts.edu/nutrition_guide
http://researchguides.library.tufts.edu/biomedical_research

**Assignment**:
1. Navigate each of the URLs in section C above to locate the 2008 *J. Nutrition* article, “Adiponectin Gene Variants Are Associated with Insulin Sensitivity in Response to Dietary Fat Consumption in Caucasian Men.” *Be prepared to summarize the last paragraph of the Discussion section in one or two sentences in class.*
2. Using Current Protocols in Bioinformatics download and read “The Importance of Biological Databases in Biological Discovery” by Baxevanis and Bateman.

**September 11: Separation / Partitioning Techniques: Gas and Liquid Chromatography**

*Learning Objectives:*

1. List the types of analytes that can and cannot be analyzed by gas chromatography and by high performance liquid chromatography.

2. Define “partition coefficient” and sketch/illustrate how elution of a solute is determined by the partitioning of a solute between mobile and stationary phases in GC and in HPLC instruments.

3. Explain the physico-chemical principles that modulate the partitioning (retention time) of a solute between phases in GC and HPLC and how these can be modified in real time to alter solute elution time. Understand the differences between “regular” phase and “reverse” phase HPLC.

4. Be able to accurately draw relative retention times and peak heights for GC and HPLC chromatograms of mixtures of i) lipids and ii) amino acids differing in relative amount, mass and/or polarity.

5. Be able to suggest appropriate alterations to chromatography conditions to optimize (reduce) “run time” and/or enhance peak shape (sharpness) for the mixtures above.

6. Be able to explain/sketch how external and internal standards are used for qualitative and quantitative analysis in GC and HPLC. Be able to explain the limitation of using standards for identifying compound(s) in samples.

*Required Reading*


http://www.youtube.com/watch?v=qOpM-k05vQQ&feature=related (2min, 19s)
http://www.youtube.com/watch?v=ffefIgLgeKx8&NR=1 (9 min, 39s)
http://www.youtube.com/watch?v=M8d1u7kFZe0 (13min, 50s)
https://www.youtube.com/watch?v=gU2st5-T1Go (26min)
http://www.youtube.com/watch?v=qOpM-k05vQQ&feature=related (2min, 19s)
https://www.youtube.com/watch?v=UuHOOlKvUbo (20min, 01s)
http://www.youtube.com/watch?v=kz_egMtdnL4&feature=related (5min, 29s)
https://www.youtube.com/watch?v=MLoitPJQH3g (3min, 23s)
**September 18: Mass Spectrometry**

*Learning Objectives:*

1. Be able to draw the basic design of a mass spectrometer, distinguishing where the following steps occur: ionization, acceleration, deflection and detection.

2. Explain how and why different ions are deflected by the magnetic field by different amounts.

3. Relate the concepts in #2 to the m/z ratio and its importance in data output.

4. Define “m+1” and “parent” ion.

5. Be able to sketch, label and explain ‘stick’ diagrams of MS output for simple compounds containing carbon, chlorine and bromine, indicating fragmentation and relative abundance. Be able to explain why each element can be distinguished by a particular pattern of fragmentation and relative abundance.

6. Describe how tandem mass spectrometry provides enhanced selectivity and discrimination in analysis.

7. Explain the explanatory power and scientific rigor that MS provides to GC and HPLC experiments.

**Required Reading/Assignment**

http://www.chemguide.co.uk/analysis/masspec/howitworks.html
http://www.chemguide.co.uk/analysis/questions/q-mshowitworks.pdf
https://www.youtube.com/watch?v=NuIH9-6Fm6U (9min, 38s)
http://www.youtube.com/watch?v=rBymrFzcaPM&feature=fvwrel (lectures 1-4; 39min, 38s)
https://www.youtube.com/watch?v=qyX7Y_3aSPI (5min, 24s)
https://en.wikipedia.org/wiki/Tandem_mass_spectrometry (Instrumentation section only)

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**September 25: Working with and Fractionating Mammalian Cells**

*Learning Objectives:*

1. Demonstrate an understanding of how to calibrate and use an air-displacement pipette:

2. Explain the sequence of steps required to aseptically feed cells in a cell culture hood, including the use of ethanol, gloves, ‘sterile’ and ‘nonsterile’ working areas within the hood, laminar flow and ultraviolet light.

3. Diagram and label the sequence of steps and reagents involved in obtaining cytoplasm, nuclei, lysosomes and highly purified ribosomes from the same cultured cells, employing both differential and buoyant density gradient centrifugation.

4. Outline an alternative and more rapid approach for obtaining a cell fraction enriched for mitochondria.

5. Be able to explain the origin and utility/importance of the Svedborg.

6. Elucidate the formula that allows reproducibility of G force among different rotors in high speed and ultracentrifugation experiments. Explain why knowledge of both the rotor brand and model and the centrifuge speed are therefore necessary to reproduce conditions among experimenters.

**Required Reading**

A. Cell Culture

https://www.youtube.com/watch?v=uEy_NGDfo_8 (8min, 48s)
http://www.youtube.com/watch?v=yl_acpKgito&feature=related (4min, 23s)
October 2: Extraction, Electrophoresis and Initial Characterization of Proteins and Nucleic Acids

**Learning Objectives:**

1. Be able to describe and justify the steps used in isolating i) proteins, ii) DNA and iii) RNA from mammalian cells using commercial kits.

2. Describe and diagram i) the structural features of native proteins that facilitate their separation in an electric field, ii) the set-up and apparatus for separating a protein mixture by gel electrophoresis and iii) the visualization and molecular weight characterization of constituent proteins.

3. Discuss the similarities and differences between In PAGE and SDS-PAGE, in particular the basis(es) upon which proteins are separated, the roles of nonionic and anionic detergents in solubilization and negative charge imposition, function of reducing agents, the principle used to optimize separation of high mass vs low mass proteins, and the function of the stacking gel.

4. Describe and diagram i) the structural features of nucleic acids that allow their separation in an electric field, ii) the set-up and apparatus for separating a mixture of DNA or RNA fragments by gel electrophoresis, iii) the visualization and molecular mass characterization of the constituents using ethidium bromide and a DNA “ladder” and iv) quantitation using a spectrophotometer. Be able to explain how and why nucleic acid fragments (as opposed to whole DNA/RNA) are analyzed in agarose gel electrophoresis.

**Required Reading**

- [https://www.youtube.com/watch?v=3CrzY7jb9fQ](https://www.youtube.com/watch?v=3CrzY7jb9fQ) (3min, 16s)
- [https://www.youtube.com/watch?v=bdBXwuwSBo](https://www.youtube.com/watch?v=bdBXwuwSBo) (13min, 50s)
- [https://www.youtube.com/watch?v=p0ZxmVbKayo](https://www.youtube.com/watch?v=p0ZxmVbKayo) (3min, 18s)
- [https://www.youtube.com/watch?v=HqmxLsHkXE](https://www.youtube.com/watch?v=HqmxLsHkXE) (2min, 15s)
- [http://www.dnalc.org/resources/animations/gelelectrophoresis.html](http://www.dnalc.org/resources/animations/gelelectrophoresis.html)
- [https://www.youtube.com/watch?v=ZuhQtTX6_4U](https://www.youtube.com/watch?v=ZuhQtTX6_4U) (3min, 8 s)
- [https://www.youtube.com/watch?v=vq759wkCCUQ](https://www.youtube.com/watch?v=vq759wkCCUQ) (4min, 7s)
- [https://www.youtube.com/watch?v=6_4AY3YRgo](https://www.youtube.com/watch?v=6_4AY3YRgo) (9min, 46s)
- [https://www.zymoresearch.com/media/amasty/amfile/attach/_D4068_D4069_Quick-DNA_Miniprep_Plus_Kit_ver_1.2.0_MT_11-29-16_.pdf](https://www.zymoresearch.com/media/amasty/amfile/attach/_D4068_D4069_Quick-DNA_Miniprep_Plus_Kit_ver_1.2.0_MT_11-29-16_.pdf) (isolation diagram on page 5 of PDF)
- [https://www.zymoresearch.com/media/amasty/amfile/attach/_R1057T_R1057_R1058_Quick-RNA_Miniprep_Plus_Kit_ver_1.0.1.pdf](https://www.zymoresearch.com/media/amasty/amfile/attach/_R1057T_R1057_R1058_Quick-RNA_Miniprep_Plus_Kit_ver_1.0.1.pdf) (pages 4-6 focusing on RNA from cells)

October 16: Antibody and Fluorescence-Based Techniques

**Learning Objectives:**

1. Diagram an antibody-antigen complex, labeling heavy and light chains and the constant and variable domains.
2. Define “epitope” and “affinity” and explain the underlying structural molecular features of antibody and antigen that determine them.

3. Describe the key molecular and practical (i.e., experimentation) differences between monoclonal and polyclonal antibodies.

4. Devise and diagram a controlled clinical trial designed to test the hypothesis that dietary fish oil supplementation reduces systemic inflammation, using circulating levels of the proinflammatory cytokine interleukin 1 (IL-1) as a read-out of systemic inflammation.

5. Identify the additional reagents that must be developed/used to measure the amount of protein X in human tears using an IgG2 monoclonal antibody (Mab) that is not enzyme-linked.

6. Diagram i) the three-step process that results in emission of light from a fluorophore, ii) what accounts for the color emitted by a fluorophore, iii) the concept of excitation and emission maxima, iv) the important functional difference between tungsten and laser excitation sources, and v) the difference and uses of bandpass filters for optimizing excitation and emission.

7. Be able to draw a labelled flow cytometry plot showing populations of large cells with agranular cytoplasm and small, granular cells.

8. Be able to explain and illustrate spectral overload and compensation in flow cytometry experiments.

9. Design and diagram the data output from a flow cytometry experiment that demonstrates that dietary lycopene supplementation in carcinogen-exposed mice significantly reduces the number of precancerous white blood cells (WBCs), which express antigen “Pr” on their surface, relative to the number of healthy WBCs, which express antigen “H” but not antigen “Pr” on their surface. Assume that you are given the blood after it is drawn from the mice. Specify fluorophores and wavelengths in your experimental design. Draw plots that indicate that the transition from healthy to precancerous involves i) loss of antigen “H” and ii) no loss of antigen “H.”

Required Reading
A. Measuring Antigens in Biological Samples (blood, tissue homogenates, etc.)
https://www.youtube.com/watch?v=dTbOiEUS1oA (6min, 12s)
http://www.dnatube.com/video/2220/Antibodies (2min, 39s)
http://www.abcam.com/protocols/a-comparison-between-polyclonal-and-monoclonal
http://www.dnatube.com/video/280/ELISA-Enzyme-Linked-ImmunoabSorbant-Assay (59s)
http://www.sumanasinc.com/webcontent/animations/content/ELISA.html (3min 40s)

B. Fluorescence and Flow Cytometry
http://www.invitrogen.com/site/us/en/home/support/Tutorials.html (5 listed tutorials; 45min, 45s for initial viewing)

October 23: Imaging Techniques (Microscopy and In Vivo Imaging)
Learning Objectives:
1. Be able to describe and/or diagram the following: advantage of using transmitted light vs fluorescent light in microscopy; the features of a specimen that determine the best fluorescent technique to use; the two general classes of solutions to the essential problem with traditional epifluorescence microscopy and the advantages and disadvantages of each.

2. Describe the features, advantages and limitations of different types of confocal microscopy, including i) how scanning is accomplished in line scanning confocal microscopy, ii) what problem(s) inherent in standard pinhole-based confocal...
microscopy are overcome by Spinning Disk Confocal, and iii) how two- or multiphoton confocal microscopy works and why is this an advantage for very thick specimens.

3. Distinguish the type of optical scanning microscopy that is best-suited for imaging the following:
   ion channels at the cell surface?
cell structures at 75 microns depth?
live cells structures at 20 microns depth?
specimens > 200 microns?

4. Briefly state why Image J and similar software is vital in microscopy.

5. Describe how and why we might employ PET-CT in nutrition research. Distinguish between the data do we obtain from PET from those we get from CT.

6. List and elucidate how other imaging techniques can be used in combination with and in support of PET-CT data to obtain experimental rigor.

Required Reading

https://www.youtube.com/watch?v=01v2kR8dlnQ (29min 7s)
http://www.youtube.com/watch?v=OH2GFeaGV6w (2min, 56s)
http://media.pearsoncmg.com/bc/bc_campbell_genomics_2/medialib/method/IMF.html
http://olympus.magnet.fsu.edu/primer/techniques/confocal/confocalintro.html (first three paragraphs)
https://www.youtube.com/watch?v=YRQsjPAx9UU (26min, 40s)
http://circ.ahajournals.org/content/117/3/379.full.pdf+html (introduction and figures 3-7)

October 30: Measuring Gene Expression

Learning Objectives:
1. Be able to draw the first four cycles of a standard PCR reaction, indicating target sequence and direction of replication for all strands. For the first cycle indicate temperatures, enzymes dNTPs and primers. What key steps must the PCR hardware be able to perform?

2. Demonstrate and understanding of RT-PCR by i) explaining the function of the key enzyme that allows us to adapt PCR for examining gene expression, ii) diagraming an RT-PCR reaction through RT and first PCR cycles, identifying enzymes and reagents used, iii) describing how we assess mRNA expression levels after RT-PCR, and iv) explaining the semi-quantitative nature of RT-PCR with a diagram of amplification vs cycle.

3. Elucidate the key advantage real-time PCR provide over standard PCR and the advantage real-time RT-PCR offer over standard RT-PCR. Explain what capability of the PCR thermocycler is required to allow for “real time” measurement of amplicons.

4. Explain/diagram the function and advantages/disadvantages of Cyber/Syber Green vs Taqman-based PCR.

5. Be able to draw and explain in quantitative detail a real-time PCR data plot of standards containing 10 ng, 20ng, 100ng and 1 microgram of hexokinase mRNA. Label the axes and accurately label the Ct differences among the samples.

6. Explain the method of calculation that is used to accurately quantitate the fold-difference in mRNA between two or more samples in a real-time PCR experiment, identifying the role of reference or “housekeeping” genes. Be able to write out the details of the ‘deltas’ in the formula, $2\Delta\Delta C_t$. 

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7. Explain how standard RT-PCR and QPCR is used in gene chips and microarray experiments to analyze a sample or a test sample vs control sample for the expression of up to thousands of genes at once. Be able to sketch a gene chip and diagram a microarray experiment comparing gene expression between control muscle cells and those treated with vitamin D. Briefly contrast these approaches with RNA-seq (i.e., the major difference/advantages).

Required Reading

A. PCR-based approaches: PCR, RT-PCR and Real-Time PCR.

https://www.youtube.com/watch?v=54kN88U4Y6w (2min, 19s)
http://www.dnalc.org/ddnalc/resources/pcr.html
http://www.bio.davidson.edu/courses/immunology/Flash/RT_PCR.html
https://www.youtube.com/watch?v=DH7o9Df5_50 (28min, 52s)
http://www.bio.davidson.edu/courses/immunology/Flash/RT_PCR.html
https://www.youtube.com/watch?v=0fEqfIiV53w&t=18s (4min, 18s)
https://www.youtube.com/watch?v=tgp4bbnj-ng (8min, 20s)
https://www.youtube.com/watch?v=GDLPVm7fglc (6min, 21s)

B. High(er) Throughput Methods (microarrays and RNA-seq)

https://www.youtube.com/watch?v=0ATUjAxNf6U (4min, 22s)
https://www.illumina.com/techniques/sequencing/rna-sequencing.html

November 6: Manipulating Gene Expression I (Transformation, Transfection, Promoter Assays, RNAi, Optogenetics)

Learning Objectives:

1. Be able to sketch and describe the work-flow of a bacterial transformation using plasmids and competent bacterial cells. Sketch a ‘basic’ plasmid; identify and explain the rationale for the origin of replication, bacterial resistance gene and LacZ gene.

2. Be able to briefly describe three methods of transfecting eukaryotic cells without viral vectors. What is the fundamental problem that each of these approaches seeks to overcome regarding introducing DNA/RNA into cells?

3. List the advantage and disadvantage of viral vectors as compared with plasmids and the advantage and disadvantage of the following viral vectors relative to each other: Adenoviruses; Adeno-Associated Virus (AAV); Retroviruses (e.g. MMLV); Lentiviruses (e.g. HIV-1, FIV, SIV)

4. Be able to diagram and explain in detail an experiment that you design to identify (“map”) the sterol response element (SRE) of a new (fully sequenced) gene believed to be regulated by cholesterol, given the following background information: Changes in cholesterol metabolism are due, at least in part, to the effect of cholesterol on specific transcription factors called sterol regulatory element binding proteins (SREBP). SREBPs bind at specific cis-regulatory elements called sterol regulatory elements (SRE) in the promoters of several genes involved cholesterol synthesis, cholesterol uptake, and fatty acid synthesis. Under low cholesterol conditions, cellular SREBP levels increase, and binding of SREBP to SRE induces transcription of these genes. Under high cholesterol conditions, levels of SREBP decrease and transcription is downregulated. Include appropriate controls for assay-to-assay variability in your experimental design.

5. Describe the intra-cellular trafficking, function and evolutionary role of RNAi
6. Diagram the critical structural feature shared by microRNA and siRNA that allows their recognition by DICER. Explain the functional difference that distinguishes miRNAs from siRNAs. Be able to clearly diagram and explain the roles of Dicer, Argonaut, guide strand and RISC complex.

7. Explain the molecular mechanism underlying optogenetics and the ‘engineering’ that establishes its use in vivo.

Required Reading

A. Bacterial Transformation
https://www.addgene.org/protocols/bacterial-transformation/
https://www.youtube.com/watch?v=cVKsVnE3rYU (5min, 19s)

B. Transfection
https://www.promega.com/resources/multimedia/reporter-assays-and-transfection/introduction-to-transfection/ (3min, 34s)
http://www.cellbiolabs.com/viral-expression
https://en.wikipedia.org/wiki/Viral_vector

C. Reporter Assays
https://www.youtube.com/watch?v=PD_6JU3NayE (2min, 16s)
https://www.youtube.com/watch?v=yQXdpwZmwnA
https://www.jove.com/video/51719/massively-parallel-reporter-assays-in-cultured-mammalian-cells (11min, 3s; admittedly advanced; just appreciate the power of this assay, how techniques that you already have covered are used, and be able to discuss the rationale – i.e., Fig. 1)

D. RNA Interference
https://www-tc.pbs.org/wgbh/nova/assets/swf/1/rnai-explained/02-expl.swf
http://www.nature.com/focus/rnai/animations/animation(animation.html (animation 5min, 7s and slide module 3)
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC1838705/ (Abstract only)
http://www.earthisland.org/journal/index.php/elist/eListRead/a_very_different_kind_of_gmo/

E. Optogenetics
http://www.youtube.com/watch?v=QA67v4vSgo0&feature=player_embedded (4min, 50s)

November 13: Manipulating Gene Expression II: (Genome Editing)
Learning Objectives:
1. Be able to diagram the CRISPR-Cas9 system within a cell, identifying the features that are responsible for its name. Identify components that are identical and the origin of the unique sequences. Describe the enzymatic activities conferred on the CRISPR-Cas9 system by the CAS gene(s).

2. Explain how the system acts in bacterial immunity, identifying similarities/differences with respect to RNAi.

3. Explain the functional importance of palindromic sequences in CRISPR-Cas9.

4. Diagram the “dual RNA-guided dsDNA endonuclease” actions of Cas9.

5. Explain and diagram the modification to the ‘original’ CRISPR-Cas9 system that produced the Nobel Prize-worthy genome-editing capability that we now associate with CRISPR-Cas9. What two RNAs comprise the guide strand or gRNA?
6. Diagram the two types of repair of dsDNA breaks, identifying the one type that is essential for genome editing using CRISPR-Cas9?

7. Describe the essential difference (and thus great advantage) between CRISPR-Cas9 and ZNF- and TALEN- based genome targeting technologies.

8. Explain \( i \) how CRISPR-Cas9 reduces/removes the potential for creating allergens in genetically-modified food, and \( ii \) how Cis-genesis differ from trans-genesis and potentially provides a mechanism for generating non-GMO golden rice.

**Required Reading**

https://www.youtube.com/watch?v=2pp17E4E-O8 (4min, 12s)
https://www.youtube.com/watch?v=MnYppmstxIs (7min, 20s)
https://www.youtube.com/watch?v=SuAxDVBt7kQ (16min, 41s)
https://www.youtube.com/watch?v=J8p0JoF1utE (30min, 52s)

**November 20: “Oms,” High Throuput Techniques and Systems Biology**

*Learning Objectives:*

1. Define ‘omics’ and list the factors driving its development and ‘omics’ fields that are currently particularly attractive to nutrition researchers.

2. Explain how ‘omics’ studies appear to be “reductionist” yet at the same time, not “mechanism-driven.” Describe how ‘systems biology’ can be a powerful adjunct to ‘omics’ approaches with specific reference to nutrition science.

3. Be able to sketch Sanger sequencing, understand how the data (A,T,G,C) are used to reconstruct the sequence and understand what limits the speed of this type of sequencing.

4. Identify the common features of the workflow for all NGS technologies. Explain what a “library” is and how they are constructed using amplification and/or ligation with custom adaptor sequences. Name the feature of NGS technology that is an adaptation and advance over traditional microarray technology.

5. Be able to define the role of: adaptor, linker, bridge PCR, emulsion PCR, cluster and describe the raw data generated by a sequencing machine.

6. Define “coverage” and explain why 30x coverage considered necessary for accurate NGS of a whole genome? Discuss types of analyses that might require greater coverage and explain why.

7. Describe and diagram RNAseq and contrast it with gene chips or microarrays for measuring gene expression.

8. Describe and diagram bisulfite sequencing, how it is combined with NGS, and what it tells us.

9. Describe and diagram Chromatin Immunoprecipitation (ChIP) and what ChIP-Seq can tell us.

10. Explain what 16s rRNA sequencing tell us, its use, and its distinction from “shotgun metagenomics?”

11. Explain the essential principle of Bayes Theorem, how this distinguishes the theorem from ‘traditional’ probability theory, and why the theorem is so vital to the emergence of NGS and other high throuput technologies.

**Required Reading**

A. Omics
“Nut225-omics-slides” (this PDF is available in Canvas under “Files” in a folder titled, “Week 11 additional reading.”)

B. Next Generation Sequencing
https://www.youtube.com/watch?v=ToKUGz_YhC4 (4 min)
https://www.youtube.com/watch?v=jFCD8Q6qSTM (9 min, 29 s)
https://www.illumina.com/techniques/sequencing.html (cover DNA, RNA and Methylation sequencing methods; understand the general steps in each “workflow.”)
https://www.youtube.com/watch?v=kTcFZxP6kM (12 min, 52 s)
https://www.youtube.com/watch?v=9vNBYyHedLg (through the first 49 min)
https://www.illumina.com/techniques/sequencing/dna-sequencing/chip-seq.html
https://www.illumina.com/areas-of-interest/microbiology/microbial-sequencing-methods.html
https://www.illumina.com/science/education/sequencing-coverage.html
https://en.wikipedia.org/wiki/Coverage_(genetics)
https://www.illumina.com/documents/seminars/presentations/2010-06_sq_04_townley_ngs_analysis.pdf (focus on image analysis, base calling and alignment to get a sense of the critical computational underpinnings of NGS).
http://www.nature.com.ezproxy.library.tufts.edu/nmeth/journal/v6/n11s/full/nmeth.1376.html
(read up to section on “Alignment”)

C. Bayesian Methods

D. Systems Biology
https://irp.nih.gov/catalyst/v19i6/systems-biology-as-defined-by-nih (refresher from week #1)
Example: Systems Biology Approach to the Link Between Obesity and Colorectal Cancer
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4540493/ (focus on Methods and Analysis)

November 27: Informatics; Computational Biology, Bioengineering, Synthetic Biology

Learning Objectives:
1. Define the types of informatics associated with the study of molecules and cells, individual patients, and populations, respectively.

2. Define “computational biology” emphasizing both the role and goal of model building and the distinction between computational biology and bioinformatics. Explain by way of example how the two fields are often functionally integrated.

3. Be able to describe the role of active machine learning in the new research paradigm of computational “model improvement” vs “model testing.”

4. Explain the rationale behind the approach and work-flow used in the assigned article, “Network analysis and data mining in food science: the emergence of computational gastronomy.” Be able to interpret the graphical network analysis presented.

5. Be able to use a Bayesian approach to determine the probability of truly having a disease knowing the percent of the population that actually has the disease and the reliability of the test for the disease that is available.

6. Design an ‘organ-on-chip’ that would allow you to determine i) if intestinal cells (enterocytes) allow passage of gut bacteria into the blood following exposure to meals high in saturated fat, and ii) if the passage of gut bacteria across enterocytes causes inflammation in fat (adipose tissue).
7. Explain the value (if any) of constructing accurate *in silico* replicates of biological systems.

8. Support or critique the value inherent in notion that *in silico* biology represents a new biological paradigm combining systems biology and engineering formalisms to design novel biological components/entities that do not exist in nature.

**Required Reading**

A. **Biomedical Informatics**
   
   [https://www.amia.org/about-amia/science-informatics](https://www.amia.org/about-amia/science-informatics)
   

B. **Computational Biology/Bioinformatics**
   

   [A-Bayesian-Networks-Approach-for-Predicting-Protein-Protein-Interactions-from-Genomic-Data.pdf](http://www.cbd.cmu.edu/about-us/what-is-computational-biology/) (included in “files” on Canvas. Read through first paragraph of page 2)


   [https://www.ncbi.nlm.nih.gov/ezproxy.library.tufts.edu/pubmed/24786325](https://www.ncbi.nlm.nih.gov/ezproxy.library.tufts.edu/pubmed/24786325) (Introduction, Methods and Figure 2).


C. **Bioengineering and Synthetic Biology**
   


   [https://www.ted.com/talks/geraldine_hamilton_body_parts_on_a_chip#](https://www.ted.com/talks/geraldine_hamilton_body_parts_on_a_chip#) (13min, 23s)

   [https://www.nature.com/articles/srep42296](https://www.nature.com/articles/srep42296) (Introduction and figures 1-3)

   [https://www.ted.com/talks/andras_forgacs_leather_and_meat_without_killing_animals](https://www.ted.com/talks/andras_forgacs_leather_and_meat_without_killing_animals) (8min,59s)