BCH 390 Research Methods in Biochemistry

An RNA Seq Module for **GCAT-SEEK**

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BCH 390 Research Methods in Biochemistry

- 300 level integrated lab/lecture
- 1 hour lecture + 4 hour lab per week
- Parallel methods with each pair testing different hypotheses
- Designed to give students independent research experience with expected outside time commitment
- Pre-requisites:
  - Intro BIO (BIO 150 Cells, Physiology, and Development)
  - Intro CHM (Chm 111)
  - Intermediate leve l genetics (BIO 230 Genomes and Genetic Analysis)

Course Introduction: Microbial Responses to their Environment

-Introduce broad possibilities with distinct framework example

-Use temperature as specific central example from which students can begin to frame their own question.

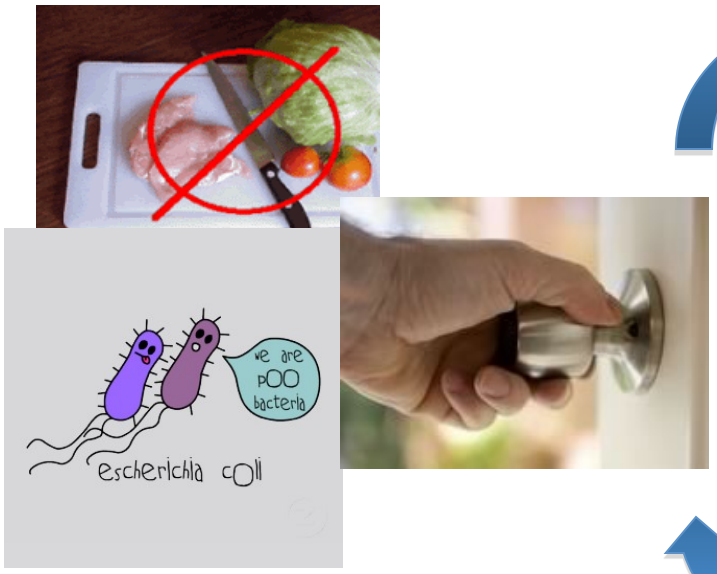
-Can discuss key concepts and utilize important bioinformatic tools within the example

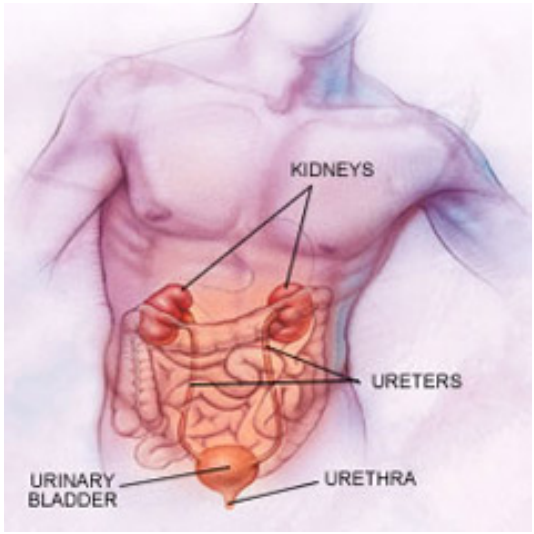
- Basic microbial genome and transcriptome organization
- Gene expression mechanisms
- Effect of varying conditions on gene expression

How do bacteria sense and respond to temperature?

23˚C

37˚C





An example project: How do bacteria sense and respond to temperature?

Framework Example /Specific Aims

1. What genes are more highly expressed at a given temperature? How do they help with host colonization or external resistance?

2. How does growth medium impact thermoregulation?

3. Are thermoregulatory responses conserved between commensal and pathogenic *E. coli*?

4. What genes are controlled by the sigma factor RpoS ?

Lecture/discussion goals

- Devise own experiment (V &C 1, 6)
  - Identify a variable that impacts microbial adaptation
  - "Big picture" significance/application
- Do literature research to support choice/define hypotheses (V &C 1, 6)
- Define specific question and develop specific experimental design to test (V &C 1)
- Write proposal and present to class (V &C 1, 5)
- Learn about and implement techniques weekly (V &C 1-6)
  - Wet lab
  - Computational analyses – Any computer with good internet connection should suffice.
- Use functional genomic resources (V &C 1, 2, 3)
  - NCBI, Ecocyc, ASAP, Regulon DB
- Write draft manuscript of experimental results with peer review (V &C 5, 6)

Lab Workflow

Week

1. Student devise own project in pairs/Literature review/ Hypotheses
2. Grow microbial samples
3. Isolate RNA
4. Isolate RNA (Cont'd)
5. RNA quality/rRNA removal
6. Construct library
7. Construct library (Cont'd)
8. MiSeq demo/Monitor run QC in real time on BaseSpace cloud
9. Learn Galaxy/QC, trim and set up assembly of output data: FASTQ
10. Run TopHat, CuffLinks and CuffDiff to quantify transcripts
11. Data analysis – invesitgation of genes of interest determined from CuffDiff output – ontology and pathway analysis
12. Data analyisis (Cont'd)
13. Presentation of data

Lecture to cover concepts behind the techniques of the lab each week as well as have students present and trouble shoot in lab meeting type discussions. For example, the first lecture will cover the intro material and example project covered in the previous

Assessment

- Before and after survey:
  - Molecular techniques
  - Gene expression
  - Scientific method
  - Confidence with various techniques to be covered
- Mini grant/project proposal (2-3 page)
- Manuscript draft
- Oral presentation