BE/APh 162: Winter 2009
Syllabus

Professor: Rob Phillips
TAs: Dave Wu, Damien Soghoian, Arbel Tadmor, Hannah Tuson

Mondays, 2-5 PM & Tuesdays, 7-10 PM in 040 Keck

Week 1 –Size of things

Session 1- Logistics and microscopy fundamentals

- Introduction to the course
- Microscopy fundamentals & optics demonstration
- Köhler illumination
- Oil/water/air objectives (demo)
    - CCD
- How it works
- Signal quantization and saturation
- Pixel resolution
    - Micromanager

Session 2– Size of things using microscopy

- Light/phase/fluorescent microscopy
    - Good practices
    - Techniques
- Making agarose pads
- Calibration of microscope using calibration slides
- Light/phase microscopy
- E. coli grown overnight in poor vs. rich media (Schaechter et al. observation)
- Look at an assortment of live bacteria and Eukaryotes

- Fluorescent microscopy
  - YFP labeled phages attached to E. coli
  - FM dye stained cyanobacteria membranes
  - Various pre-stained slides

Week 2 – Rate of things

Session 1– Rate of things using microscopy

- Light/phase microscopy
  - Movie of E. coli growth
  - Movie of yeast growth
  - Beating of Chlamy cilia
  - Dictyostelium
- Fluorescent microscopy
  - Photobleaching of fluorescent E. coli cells

Session 2 – Rate of things using spectrophotometry

- Theory
  - Diauxic growth curve using different sugars
    - Operons and operon regulation
    - Order of magnitude estimation
  - Spectrophotometry
    - Beer-Lambert law
    - Demonstration on chlorophyll
    - OD_{600} vs. cfus

- Experimental
  - Measure diauxic growth curve
    - 1:3 Glucose:Lactose
    - 1:3 Glucose:Arabinose
    - 1:3 Glucose:Sorbitol
    - 1:3 Glucose:Maltose
  - Plate cells

Week 3 & 4 – DNA engineering

Session 1– PCR

- Outline experiment
- PCR YFP insert
  - PCR protocol
  - Proper pipetting techniques
  - Execute PCR
- Restriction digest
  - What we are digesting and why
  - How to predict cutting sites (internet/Vector NTI)
- Project discussion

Session 2 – Restriction digests and gels

- PCR purification of insert
- Digestion
  - Insert
  - Plasmid
  - Lambda
- Gel electrophoresis
  - Cast gels
  - Talk about gel electrophoresis
  - Set up samples for gel
  - Load gel
- PCR purification of digested insert

Session 3 – Ligation and transformation

- Outline for today
- Nanodrop PCR insert

- Ligation
  - Figure out volumes
  - Set up reaction
- PCR purification of ligation product
- Transformation
- Plating

Session 4– Analysis of transformed cells

- Inspect transformed cells under microscope
- YFP induction as a function of IPTG
- YFP induction as a function of looping
- YFP induction for cells with mutant operators
- Measuring expression from Hernan's YFP/CFP strains
- Novick & Weiner single-cell version using fluorescence (Van Oudenaarden paper)

Weeks 5-9 – Projects

Week 10 – Project presentations