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