ESRM 404: PLANT MICROBIOLOGY LAB DRAFT SYLLABUS FOR 2019
Professor Sharon Lafferty Doty, University of Washington

WEEK 1 (JAN 7 – 11)

Monday

1. Pipette techniques
2. Sterile techniques
3. Pouring agar media plates
4. Streaking technique
5. Introduce and assign course isolates
6. Streak isolates on MG/L for single colony selection
7. Prepare pots with soil and labels for plant experiment

Wednesday

1. Morphological description of isolates, plates and microscope
2. Light microscope, wet mount of isolates
3. Transplant treatment and control plants to potted soils

Friday

1. Streak isolates on selective media
    a. N, P, Siderophore
    b. antibiotics

WEEK 2 (JAN 14 – 18)

Monday

1. Streak isolates on MG/L for single colony selection
2. Check selective media and plates for results
3. Screen isolates for antibiotic resistance

Wednesday

1. Prepare cell cultures for inoculum

Friday

1. Time zero measurements of plants, part 1
2. Measure OD on spectrophotometer, and prepare inoculum
3. Inoculate seedlings

WEEK 3 (JAN 21 – 25)

Monday **MLK Jr. Day (No class)**

Wednesday 23

1. Mix 50 ul each of the pre-made cultures of donor, helper, and your recipient strain
2. Dispense the 150 ul mixture onto centers of MGL plates
3. Time zero measurements of plants, part 2
4. Using the colonies from Jan 11 N-free plates, and using sterile technique, collect the cells in an Eppendorf tube with 100 ul NF-CCM broth. Vortex and transfer all to ARA vials containing NF-CCM agar. Dose with acetylene gas (Note: highly flammable gas)

Friday 25

1. Plant Data, W1; water with specific volume of 1/10X Hoaglands Solution
2. Conjugation experiment: Scrape up the conjugated cells and streak onto appropriate selective medium
3. For ARA: Remove 5 ml air from CLEAR AUTOSAMPLER VIALS. Then replace with 5ml air from your ACETYLENE-DOSED AMBER VIALS. These will be run on the GC-FID on Monday, and you will see the data and instrument on Wednesday.

WEEK 4 (JAN 28 – Feb 1)

Monday 28

1. Check results of transconjugants streaking; re-streak on antibiotic plates to purify (label as strain name plus the marker)
2. Lecture on plant data analysis tools

Wednesday [timing: start with cultures and ARA prep; as groups of about 10 finish, go with Robert to WFS216; can split there between microscope (Robert) and GC-FID (Andrew)]

1. Small group trips to WFS216 to view on epifluorescent microscope
2. Restreak transconjugants
3. ARA: Field trip to WFS216 includes Andrew Sher tour of the GC-FID
4. Oral presentations about the strain characterizations

Friday 2/1

1. Plant Data, W2; water with specific volume of 1/10X Hoaglands Solution

2. Restreak transconjugants

3. Streak wild-type strains onto MGL for colony PCR. Lecture on molecular biology

WEEK 5 (Feb 4 – 8)

Monday

1. Colony PCR
2. Start cultures of transconjugants in antibiotics to make perms (cryogenic stocks)

Wednesday

1. Electrophoresis of some of the sample to check
2. While gel is running, do ExoSAP-IT
3. Mix tagged strain cultures with 50% glycerol and store in dry ice [concludes this experiment]

Friday

1. Plant Data, W3; water with specific volume of 1/10X Hoaglands Solution
2. Prepare sequencing reactions

WEEK 6 (Feb 11 – 15)

Monday

1. BLAST (NCBI) search of PCR results (80 min)
2. Sequence alignments in MEGA (80 min)
3. Class discussion of data collected so far

Wednesday

1. ImageJ tutorial

Friday

1. Plant Data, W4; water with specific volume of 1/10X Hoaglands Solution (45 min)
2. Discussion of "take home" project about collecting plants for endophyte extraction; students decide what selection plates they will need for Wednesday

WEEK 7 (Feb 18 – 22)

Monday **Presidents Day (No Class)**

Wednesday

1. Endophyte extraction and plating on selective medium

Friday

1. Plant Data, W5; water with specific volume of 1/10X Hoaglands Solution (30 min)
2. Look at plates (probably no colonies yet, depending on selection)

WEEK 8 (Feb 25 – Mar 1)

Monday

1. Analysis of results from endophyte extractions. Discuss. This concludes this experiment

Wednesday

1. Chlorophyll via SPAD
2. Discuss LICOR and other photosynthesis measurements but direct to EcoPhys labs for learning how to do these

Friday

1. Plant Data, W6; water with specific volume of 1/10X Hoaglands Solution

WEEK 9 (Mar 4 – 8)

Monday

2. Thorough discussion of plan for Wednesday

Wednesday

1. Harvest plants, take final measurements, and set to dry

2. Photograph plant tissue for ImageJ

Friday

1. Weigh dried plant tissue, record data

WEEK 10 (Mar 11 – 15)

Monday

1. Compile data from experiments

Wednesday

1. Statistical analysis techniques for the data

Friday

1. Final Reports Due