

MOLECULAR BIOLOGY 380.01 GENERAL OUTLINE OF LAB SCHEDULE

Note that "**Day 1**" refers to your regularly scheduled lab time. "Lab group" refers to you and your partner(s).

Week of January 22

Day 1:

1. Introduction to first lab project
2. Study and sign safety sheets
3. Lab bench sign out
4. Test phenotype of strains. Confirm drug sensitivities of DH5 α (pKOK6) (Amp^RKn^R), LGJ14 (Amp^SKn^R), and your designated test strain (Kn^R, may or may not be Amp^R).
5. Streak stock plates of above strains.
6. As a class, prepare 10ml of 25mg/ml kanamycin and 10ml of 50mg/ml ampicillin in ddH₂O. Filter sterilize and aliquot approximately 1.5 ml (into sterile microfuge tube) of each antibiotic per lab group. In liquid media, use ampicillin at a final concentration of 100 μ g/ml and use kanamycin at a final concentration of 50 μ g/ml for *E. coli* cultures and 25 μ g/ml for *Acinetobacter* cultures. Store at 4°C in the dark.
7. Make solutions for mini-prep isolation of plasmid DNA (section H) and 5MNaCl, 10% SDS (see me) and CTAB/NaCl (see me) solution for isolation of genomic DNA (section M).

Day 2:

1. Check and record phenotypes. Store stock plates at 4°C, wrapped in parafilm.

Week of January 29

Day -1

1. Begin overnight cultures (2 of each per group) of DH5 α (pKOK6), LGJ14, and your test strain in L-broth + appropriate antibiotics.

Day 1

1. Isolation and digestion of chromosomal LGJ14 DNA (6 tubes/group) and your test strain DNA (6 tubes/group). During your extractions, combine 2 tubes of identical samples, if/when the volume of your aqueous sample (containing your DNA) decreases by about half. Within each group, **combine** tubes of same strains **before** digestion.

2. Isolation and digestion of pKOK6 DNA using mini-prep procedure (rapid plasmid isolation, 1-2 tubes/group recommended).
3. Prepare 5X TBE for agarose gels.

Week of February 5

1. Run digested DNA on an agarose gel and confirm restriction map of plasmids. Also compare digested to undigested chromosomal DNA.
2. Prepare solutions for blotting.

Week of February 12

1. Run gels and set up blot.
2. Label probe (pKOK6).
3. Prepare solutions and organize materials for hybridization.
4. Initial project consultations

Week of February 19

Day -1

Prehybridization and hybridization.

Day 1

Wash membrane and prepare solutions for chemiluminescent detection.

Week of February 26

1. Detection and analysis of hybridizations.
2. Bibliography for project proposals is due.

Week of March 5

1. Catch-up.
2. Project design.
3. Tentative field trip.

Week of March 12

1. Begin projects, officially!
2. Formal project proposal is due.
3. If you plan on using primers for your project, the sequences are due and must be approved BEFORE you leave lab.

Week of April 2

Lab meeting/progress reports during lecture time

Week of April 30

1. Last day to work on projects!
2. Conferences on final projects.

Week of May 7

Lab clean-up and check out

Thursday May 14

Project paper is due before class.