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 21

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 DH5α(pJH1) (Amp\(^8\)Kn\(^5\)), LGJ14-D (Amp\(^5\)Kn\(^8\)), and ADP1 (Amp\(^8\)Kn\(^5\)).
5. Streak stock plates of above strains.
6. As a class, prepare 10ml of 25mg/ml kanamycin and 10ml of 50mg/ml ampicillin in ddH\(_2\)O. Filter sterilize and aliquot approximately 1.5 ml (into sterile microfuge tube) of each antibiotic per lab group. Use ampicillin at a final concentration of 100µg/ml and use kanamycin at a final concentration of 50µg/ml for E. coli cultures and 25µg/ml for Acinetobacter cultures. Store at 4°C.
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. Store stock plates at 4°C, wrapped in parafilm.

Week of January 28

Day -1
1. Begin overnight cultures (2 of each per group) of DH5α(pJH1), LGJ14-D, and ADP1 in L-broth + appropriate antibiotics.

Day 1
1. Isolation and digestion of chromosomal ADP1 DNA (6 tubes/group) and LGJ14-D DNA (6 tubes/group). Within each group, combine tubes of same strains before digestion.
2. Isolation and digestion of pJH1 DNA using mini-prep procedure (rapid plasmid isolation, 2 tubes/group recommended).
3. Prepare 5X TBE for agarose gels.
Week of February 4

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 11

1. Run gels and set up blot.

2. Label probe (pJH1).

3. Prepare solutions and organize materials for hybridization.

Week of February 18

Day -1
Prehybridization and hybridization.

Day 1
Wash membrane and prepare solutions for chemiluminescent detection.

Week of February 25

1. Detection and analysis of hybridizations.

2. Bibliography for project proposals is due.

Week of March 4

1. Catch-up.

2. Project design.

3. Tentative field trip.

Week of March 11

1. Begin projects, officially!

2. Formal project proposal due.

3. If you plan on using primers for your project, the sequences are due.

Week of April 15
Lab meeting/progress reports during lecture time

Week of April 29

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

Week of May 6

Lab Clean-Up and Check out

Thursday May 9

Project papers due before class