Intro to project, examine AP structure & sequence, assign reading, make media & plates.

Make competent cells, transform E. coli with phoA plasmid, assign mutant homework.

Pick colonies and start 1mL cultures, turn in mutant ideas.

Isolate phoA plasmid from 1mL cultures, run DNA gel, refine mutant ideas.

Inoculate 1mL cultures for 1 Liter prep., make solutions, show how to design primers.

Harvest 1 L cultures, isolate periplasmic fraction, heat ppt., turn in primer designs. (Long Lab!)

Ammonium sulfate ppt., start dialysis, run protein assays

Run column, check activity of column fractions, assign enzyme kinetics reading/homework, finalize and order primers.

Presidents’ Day

Run protein assays on column fractions, begin enzyme kinetics assays.

Run protein gels on column fractions, continue enzyme kinetics work.

Quick change mutagenesis, PCR, turn in enzyme kinetics for wild type, compare results.

Dpn I digest, transform XL1-B cells, time for discussion/questions.

Midterm exam, pick colonies to start 1 mL cultures for mutant screening.

Isolate mutant plasmid DNA from 1mL cultures, run DNA gel, add sequencing primers to plasmids and send off for sequencing.

Make ^phoA strain competent cells and transform with mutant(?) plasmids that were sent off for sequencing.

Spring Break

Spring Break

Analyze mutant DNA sequences, start 1mL culture to begin 400mL cultures next day.

Harvest 400mL cultures, isolate periplasmic fraction, heat ppt. (Long Lab!)

Ammonium sulfate ppt., start dialysis.

Run Q-sepharose column to purify AP mutants.
M Apr 5    Check activity of column fractions, run protein assays.
W Apr 7    Run protein gels on column fraction samples.
M Apr 12   Test thermal stability of wild type AP vs mutant enzymes.
W Apr 14   Run protein assays to compare wild type and mutant kinetics, determine Vmax & Km.
M Apr 19   Test enzyme kinetics with inhibitors.
W Apr 21   Day of comparing data of all the mutants, make sure all calculation are correct.
M Apr 26   Review & discussion of all data on all mutants, time to work on paper.
W Apr 28   Final Paper due

(27 Lab Days)