

## ***Recombinant DNA***

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Office Hours: MWF 11am-1pm and by appointment

Textbook: Recombinant DNA 2<sup>nd</sup> edition, Watson et al. (required)

*We will also use the textbooks from last quarter for a few selected topics.*

Lab textbook: none, lab protocols will be provided on the class website.

Course objectives: to expose students to the basic and advanced techniques of Recombinant DNA technology as they are used in biotechnology labs today.

Lecture Topics - this is an approximate schedule of lecture material and the background reading that can be found in the textbook

| Week of        | Lecture Topic  | Background Readings   |
|----------------|--|---|
| Jan 3-7        | Introduction to Recombinant DNA Lab Project<br>- cloning and over-expression of the PhoA gene from <i>E. coli</i><br>- advanced PCR techniques     | Watson <i>Ch. 5, 6, and 7</i>   |
| Jan 10-14      | Prokaryotic and Eukaryotic gene regulation   | Watson <i>Ch 4, 9, and 20</i><br>Karp <i>Ch.12 (517-541)</i><br><i>Bb Handout</i>   |
| Jan 17-21      | <i>cont. gene regulation</i><br>- RNAi<br>- alternative splicing<br>- chromatin structure and regulation   | Karp <i>Ch. 11 (pp 466-471)</i><br><i>Ch. 12 (pp. 498-514)</i><br><i>Bb Handout</i> |
| Jan 24-28      | Exam #1<br>Analysis of Gene expression at the single gene level and genomic level<br>- Southern blots, Northern blots and Arrays                   | Watson <i>Ch. 7</i>   |
| Jan 31 - Feb 4 | <i>cont. gene expression</i><br>Cloning and expression of mammalian genes.<br>- cDNA libraries, transient transfections, plasmid and viral vectors | Watson <i>Ch.12, 23</i>   |
| Feb 7-11       | ES cell technology targeted gene replacement, Gene traps<br>Gene Therapy   | Watson <i>Ch. 25</i><br><i>Bb Handout</i>   |
| Feb 14-18      | <i>Gene Therapy cont.</i><br>Exam #2   |   |
| Feb 21-25      | Model systems<br>Yeast – two hybrid screens, synthetic lethal and overexpression screens to detect   | Watson <i>Ch. 13 and 14</i><br><i>Bb Handout</i>                                    |

|                   |   |  |
|-------------------|---|--|
|                   | genetic interactions<br>Mouse as a model systems              |  |
| Feb 28 -<br>Mar 4 | <i>Model systems continued</i><br>Start student presentations |  |
| Mar 7-11          | Student presentations   |  |
| Mar 17            | Final Exam (normal lecture time 3:30pm<br>Thursday)           |  |

Exam Dates

Exam 1 – Jan 25

Exam 2 – Feb 15

Final Exam – March 17

***Exams constitute 50% of your final grade***

Lab Notebook Inspection dates

Midterm – Feb 10

Final – March 10

*Laboratory grade constitutes 35% of your final grade*

Group Project – *constitutes 15% of your final grade*

The group project will be done very similarly to the project in the fall quarter. It is composed of two parts: a 4 page summary of your chosen research article and the experiments. I will use this to give you feedback before the second part of the project, your oral presentation. Selection of the research article will be based on slightly different criteria. Search newspapers or magazines such as the New York Times, Scientific American, Time or Newsweek (or on similar types of websites) for articles on new drug therapies, gene therapies, or any medical advance due to recent accomplishments in the fields of Molecular Biology and Recombinant DNA technology. Once you identify an article, find the primary research articles that led to this "breakthrough". You will attempt to evaluate the validity of claims made in the original article that was obviously written for mass consumption. Sometimes you will find that journalists can inflate claims made by scientists or scientists will inflate claims to journalists to help their individual careers or their companies. Each group will make a 12-15 minute presentation to the class discussing their findings particularly trying to evaluate the promise of the reported "breakthrough".

***Important dates for group project***

Selection of topics and article – Jan 27

Written Reports due – Feb 17

Oral Presentations – March 3, 8, 11

***Laboratory Objectives***

The ultimate goal in this project is to clone the gene encoding for the enzyme alkaline phosphatase from *E. coli* in order to purify a large quantity of the recombinant enzyme. There are obviously several intermediate steps in this process and one objective of this laboratory course is to get each student

involved in the experimental design of each step. You may use this to organize your experimental outline. That is to say for each lab period you will identify your experimental objective and then find the appropriate protocol in the protocols folder on the Blackboard site for this course.

### ***1st Section - Generate basic components for cloning***

- 1) Purify the bacterial expression vector pET22b from an E. coli strain carrying the plasmid.
  - *each group will be given a saturated bacterial culture*
  - *utilize the promega wizard prep protocol to purify the plasmid.*
- 2) Isolate the PhoA gene from E.coli using PCR amplification.
  - *the PCR protocol outlines conditions and primer sequences*
  - *purify the DNA from the PCR reaction using QIAEX beads*
- 3) Use restriction enzymes to correctly identify that the plasmid and PCR fragment.
  - *using the restriction maps for the PhoA gene and the pET22b vector, identify an enzyme digest to determine if you isolated the correct pieces of DNA.*

### ***2nd Section - Cloning the PhoA gene into the pET22b vector***

- 1) Digest pET22b and PhoA PCR fragment with NdeI and XhoI
  - *each piece of DNA can digested sequentially by NdeI then XhoI or as a double digest, but be careful about buffer compatibility(see the chart in the Promega web site.*
- 2) "Clean up" restriction digests using QIAEX beads
- 3) Set up ligation reaction with the digested vector and PhoA gene
- 4) Transform *E. coli* with ligation reactions and plate then select for transformants.
- 5) Screen transformants for pET22b with the PhoA gene properly inserted.
  - *pick individual colonies from you transformed plates and inoculate 5ml LB cultures*
  - *do plasmid mini preps on your transformants*
  - *perform restriction digests to confirm the presence of the PhoA gene in pET22b.*

### ***3rd Section - Overexpression of PhoA gene and purification of Alkaline Phosphatase.***

- 1) Transform BL21 cells (*E. coli* better suited to protein overexpression) with the plasmid containing the PhoA gene.
- 2) Induce culture IPTG which stimulates overexpression of alkaline phosphatase.
- 3) Lyse bacterial cells and purify alkaline phosphatase using Ni-NTA agarose.
- 4) Measure purity of alkaline phosphatase using western blotting.