# Biochemistry Lab
# (CHEM 455)

Jung-Hyun Min, Ph.D.
Assistant Professor

## Lecture Schedule (Spring 2014)

| Week | Date | Topic |
|---|---|---|
| 1 | 1/17 | Course overview, DNA isolation and digestion |
| 2 | 1/24 | Molecular biology techniques: electrophoresis and PCR |
| 3 | 1/31 | Protein purification I: general |
| 4 | 2/7 | Protein purification II: gel filtration and ion exchange chromatography |
| 5 | 2/14 | Protein purification III: electrophoresis and enzyme assays |
| 6 | 2/21 | Protein structure: β-lactamase |
| 7 | 2/28 | Protein structure-function studies |
| 8 | 3/7 | Protein expression |
| 9 | 3/14 | Midterm Exam |
| 10 | 3/21 | No Class |
| 11 | 3/28 | Spring Break |
| 12-15 | | TBD |
| 16 | | Final Exam (Date/Time TBA, 5/5-5/9) |

## Course Overview

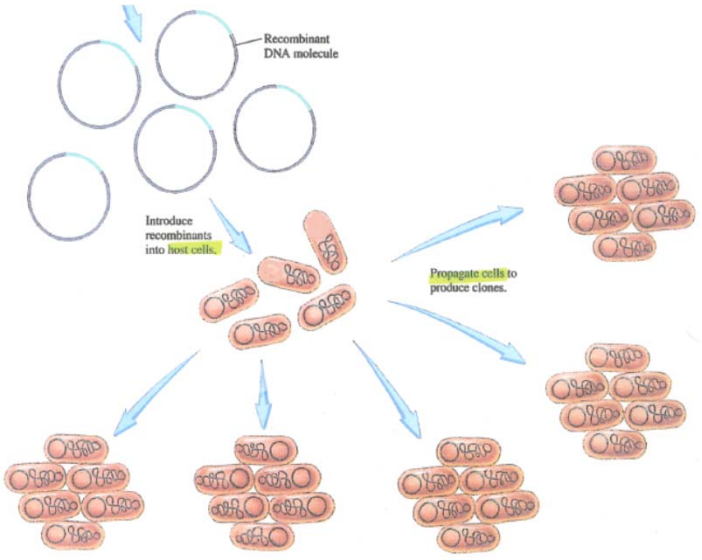
*& Why are we doing this?*

- Clone a GENE
  (i.e., *β-Lactamase*)
- Purify the gene's product, the PROTEIN
  (i.e., β-Lactamase)
- Analyze the purified PROTEIN's Structure and Function

## Glossary

- To clone a gene
  = **To create multiple copies of a gene** by growing a clone of carrier cells (such as *E.coli*) into which the gene has been introduced and from which it can be recovered **by recombinant DNA techniques**.
- Gene
  = Region of DNA that is transcribed as a single unit and carries information for a discrete hereditary characteristics, usually corresponding to (1) a single* protein or (2) a single* RNA.
  * can be a set of "related proteins/RNA.

## Molecular Cloning



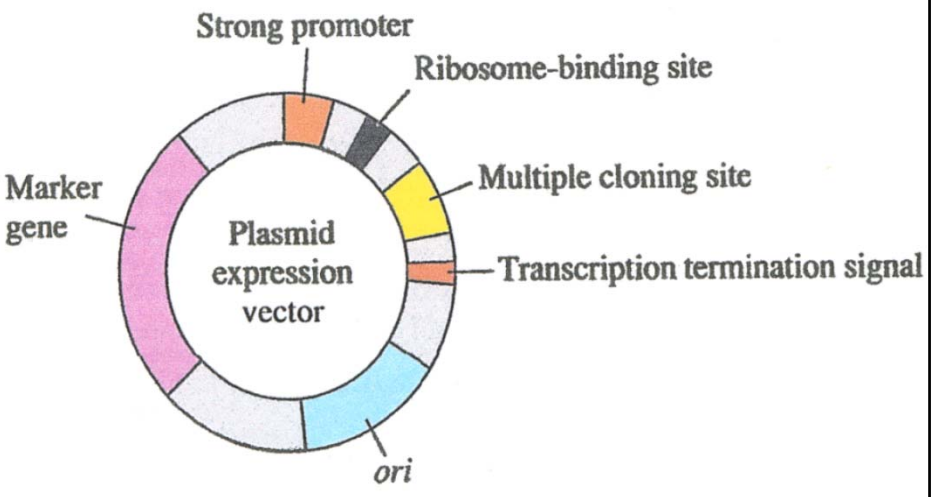

# Recombinant DNA

- Recombinant DNA
  = Any DNA molecule formed by joining DNA segments from different sources.
- DNA isolation/Restriction Enzyme digestion
- PCR
  - Mutagenesis
- Ligation
- Transformation (into *E. coli*)
  - Expression
  - Purification

# PCR

- = ?
- Required reaction components
  - E.g., polymerase, dNTPs, primers, etc.
- Primer design
  - DNA amplification, mutagenesis
- Reaction conditions

## Transformation/Expression



## Protein Purification

- Ion Exchange Chromatography
- Gel permeation Chromatography
  or Size-exclusion Chromatography

Principles

Conditions (see sample exam)

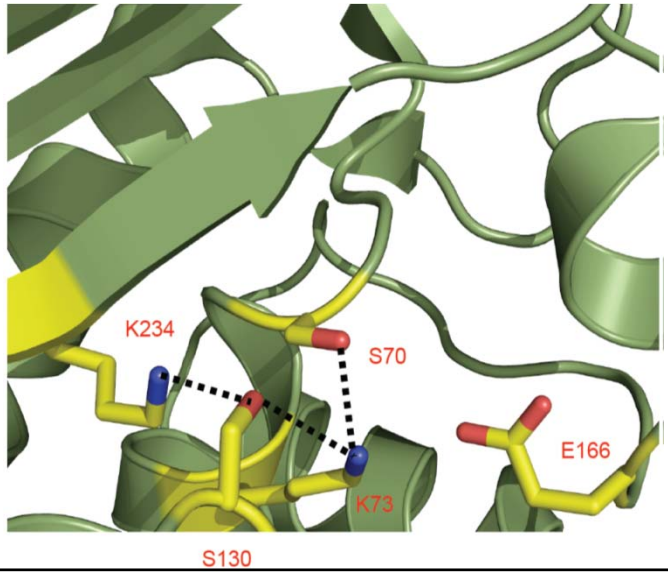
## Protein Characterization

- Electrophoresis
  - SDS-PAGE
    - Principle
    - Conditions
    - Also, agarose gel electrophoresis
- Concentration
  - BCA (bicinchoninic acid) assay
  - UV-Vis absorbance (also for DNA)

# Protein Structure

- Structural representations
  - How to interpret
  - Information content
    - E.g., how can structure be used to hypothesize a catalytic mechanism for an enzyme.

# Active Site Residues of β-Lactamase



## Enzyme Activity

- Michaelis-Menten kinetics
  - Kinetic scheme
  - Rate equation
  - Lineweaver-Burke plot
- Mechanism of beta lactamase
  - Related: How beta lactam antibiotics work, how beta lactamases lead to resistance
- Activity assay (nitrocefin)

## Structure/Function

- Mutational Analysis