7.344 Directed Evolution: Engineering Biocatalysts
Spring 2008

Session 1 Lecture Notes

1. How to read a scientific paper: (Ask how much experience they have with this.)
   a. Read with a pen in hand. Underline things you think are important. Make comments or write questions to yourself in the margins.
   b. Pay special attention to the figures. What does each figure contribute to the paper? Why did the authors think it was necessary to include it? Does the data in a particular figure substantiate the claims made in the results section of the paper? Were there unsubstantiated statements in the paper? Was there data not shown?
   c. References are there to help. Go and look up references to help you understand concepts. (Sometimes this becomes a very circular task.)
   d. Come away from the paper with a sense of what was done and try and evaluate it. Was it a meaningful or worthwhile addition to the field? (This will take some time and general understanding to say.) Think about a method's pros and cons as you read.
   e. Reread the paper. Sometimes (most of the time!) it takes several readings of a particular paper to really get it.
2. Directed evolution overview: introduce paradigm and explain its iterative nature. Explain the need to link genotype and phenotype in the screening process so that one can identify library members with desirable properties.
   a. Why do we want to engineer/evolve enzymes? To make improve their properties for some purpose (typically an in vitro application). Some examples include improving thermal stability, enantioselectivity, overall activity, changing the substrate specificity…
   b. What are the two main steps to the evolution process? Library generation and library screening. Both are important – generation of a good library should be unbiased and get at a big part of sequence space (When is this not true? If you know something about an enzyme in terms of active site residues, etc… even then it can be best.) Screening means selecting for clones capable of doing your enzymatic process – can be difficult if not linked to cell survival – why?
3. PCR – does everyone know what it is? Can someone in the class please explain? Go through slide.
4. Transitions versus transversions. When mutating DNA transitions are purine to purine changes (A to G or vice versa) or pyrimidine to pyrimidine

changes (T to C). Transversions are purine to pyrimidine substitutions and cause much more dramatic changes to the DNA.

5. What is a silent mutation? One where the change in a single base pair doesn't change the encoded protein. What is a frame shift?

6. Molecular cloning. Ask if they have any experience with this and get them to explain.