7.344 Directed Evolution: Engineering Biocatalysts
Spring 2008

Session 9 Lecture Notes

1. The authors are using a cell surface display method to screen a library of mutagenized OmpT – a bacterial cell surface protease- for new variants with either altered specificity or enhanced activity.
2. The library is formed using random mutagenesis via EP-PCR using Mn conc variation. They state that they don't know how much mutation is going to be beneficial for the protein, so they chose this method.
3. FRET stands for Forster (or fluorescence) resonance energy transfer, which may occur between two chromophores in close proximity (<10nm). This is particularly useful in detecting the products of biochemical or enzymatic reactions. Can be done with small molecules or entire proteins (cyan fluorescent protein and Yellow fluorescent protein.)
4. FACS stands for fluorescent activated cell sorting and allows for cells to be separated on the basis of fluorescent properties.
5. The authors find that their method allows for a 5000 fold catalytic enrichment in a single round of selection. They evolve novel substrate specificity – cleavage between arg and val. Major hydrolysis product was AMC meaning cleavage was happening after the valine residue. Catalytic efficiencies were much better than wild type too (60 fold for best mutant). High numbers of mutations present in clones – sometimes leads to lots of gain of function.
6. This is a nice technique for enzymes that naturally occur on the surface of cells, but potentially harder with out that as there is no display strategy presented in this paper. One benefit, screening cells by FACS allows for kinetic analysis of enzyme efficiencies. Great catalytic enrichment. Hard to translate to other substrates without a lot of linker strategies.
7. Get students to explain ice nucleation protein display system. Discuss results of display paper. Make sure that students understand the Congo red assay.
8. What is carboxymethylcellulase? Why would one want to evolve an enzyme that breaks it down? It is a beta1, 4 glucose polymer with carboxymethyl groups in place of the OHs in various positions. It is a good lubricant (artificial tears) and food additive (thickens ice cream). Adds viscosity.
9. What is the detection method for this screen?
10. How is the library generated? DNA Shuffling. Is this a good choice?
11. Explain the screening process. Page 790. Glucose liberated from the CMC hydrolysis can be used as a carbon source allowing growth on minimal media.
12. What are the results? In total 3 rounds of mutagenesis were completed and clones with useful activities were selected at each round. Are these useful

clones? What key control was performed? Western blot for expression analysis of the clones.
13. What is a key benefit? Enzymes like proteases and lipases that can be cytotoxic can be evolved this way.