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7.344 Directed Evolution: Engineering Biocatalysts □ □
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Session 3 Lecture Notes

1. DNA Shuffling – what is the method and what are the steps? Look at Figure 1. Starts with a group of enzymes that are similar or are point mutants of one another. Allows for the beneficial parts of similar enzymes to be recombined.
2. What are the results? New lactamase variants were discovered that increased the MIC of cefotaxime from 0.02 μg per ml to 640 μg per ml – much better at hydrolyzing the antibiotic!
3. How does this technique compare with others? EP-PCR (Alone) gave MIC of 0.32 μg .
4. What are the benefits to using this method? More mutations occur in each clone potentially leading to greater changes in enzymatic activity.
5. What are the pitfalls? The process is a bit lengthy.
6. StEP extension – what is the method and what are the steps? It is a recombination method that relies on the extension process of PCR (can be error prone). A family of genes can be used (as in the paper with subtilisins) as the template and recombined. Discuss figure 1.
7. What is the key to this technique? Tight control of the polymerization-catalyzed DNA extension – too much extension in each round severely limits recombination!
8. What are the results? Subtilisin was converted into a hyper-thermostable enzyme by directed evolution in vitro. 50-fold increase in enzyme half-life was achieved in two rounds.
9. Why would some mutations be too close to recombine? (Pitfalls.)
10. What are the benefits? Efficiency and benefits of other recombination methods, but can be done in a single tube without removing parent templates.
11. For next week:
 - a. Zone of clearing assay – good test of one of two things, a small molecule or bacteria producing a small molecule's ability to kill off other bacteria in the area.
 - b. Western blot – draw a schematic on the board. Get a student to explain it first.
 - c. What does an antibody's structure look like? How are antibodies produced? How do they work? (Explain affinity) Introduce terms V_h and V_l (as opposed to constant regions) and ScFV (Single chain variable fragments) is a fusion of the variable regions of an antibody using a linker.

- d. Intro to phage display. What are bacteriophage? Particles of protein that infect bacteria and turn on production of phage. Show a diagram of phage structure. What is a phagemid? Has a phage origin of replication, but is deficient in other proteins necessary for the production of phage. How can you use a phagemid as a cloning vehicle? Explain phage origin of replication and production of fusion proteins – surface exposed on phage. Phage can be selected based on antibody affinity. Explain that this is just a taste of this selection method and that we will go much more in depth. Encourage students to focus on the results in the paper relative to the mutant proteins produced.
- e. Briefly mention the technique of ELISA. Ask if anyone knows what it is and explain that we will discuss it later in the selection part of the course.