

MIT OpenCourseWare  
<http://ocw.mit.edu>

7.344 Directed Evolution: Engineering Biocatalysts □ □  
Spring 2008

For information about citing these materials or our Terms of Use, visit: <http://ocw.mit.edu/terms>.

## Session 2 Lecture Notes

1. Ask again if everyone understands PCR. Some terms to discuss: Hot start and prolonged extension. Why would one use these? Hot start inhibits polymerase activity during sample preparation thereby eliminating non-specific amplification. Prolonged extension allows for final extension to be completed. (especially if large or GC rich because  $T_m$  and annealing temp is higher.)
2. How is the gene of interest selected? Vector contains large deletions in the ribozyme gene.
3. What is the method and how is it done? Authors vary the amounts of individual bases
4. What are the results of this paper? Look at Table 1 and Figure 3. Method identified for PCR that gives an overall mutation rate of 0.66% with no strong mutational bias. What is mutational bias? Is that obvious in this procedure? (Less diversity).
5. What are the benefits to this method?
  - a. Cost effective
  - b. Randomly distributed mutations
  - c. PCR yield remains high
  - d. Reasonable mutation rate
6. Are there other ways to increase the mutation rate using this method? Increase the concentration of Taq, increase the extension time within each cycle, increase the  $MgCl_2$  concentration. How do these changes work?
7. What are the pitfalls of this method? Low mutation rate – few clones will have more than one mutation.
8. Cassette mutagenesis: What is the method and how is it conducted? Figure 1 and 2. Site for mutagenesis is introduced by putting in a site for blunt digestion. Cassette is inserted and then extra part digested out and vector re-ligated to include mutation.
9. Blue-white screening: using X-gal plates. Another way to select for clones containing a gene of interest.
10. What are the results? Any of the 20 AA may be put in at a particular site.
11. What are the benefits? 1 set of primers for all cassettes (for all possible mutants) – cost effective.
12. When would you use this method? When you know something about a particular site in a protein, i.e.—structure/function is known.

13. What are the pitfalls? Single position of site-directed mutagenesis. Slow. Can be costly because you still must make the mutant and a particular site for initial digestion.