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7.344 Directed Evolution: Engineering Biocatalysts □ □
Spring 2008

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Session 5 Lecture Notes

1. What are the authors trying to do in the aspartate aminotransferase paper? They are trying to give aspartate 2-oxoglutarate aminotransferase (AspAT) a new substrate specificity. Typically this enzyme prefers acidic substrates and aromatic substrates. They want to make it tolerate branching at the beta position. Show the picture of the aminotransferase reaction. Explain how PLP (pyridoxal phosphate) transfers amino groups (typically extracted from the gamma position of lysine residues) to the carbonyl carbon of the substrate. Why do they want to do this? Mostly just to show that they can, but one could imagine synthetic targets that would benefit from this too.
2. What process do they use to conduct the evolution? Get students to explain the process written in the materials and methods. It is very nicely done! First they have to make an auxotrophic strain that can't make any branched AAs. Then they make their library and do selection in between rounds of mutagenesis.
3. Shuffling is used for library formation – they shuffle each round of clones by picking ~90 to work from. Kind of silly up front, but makes sense after the first round. Make sure students noticed that the total number of colonies is increasing in each round, but input is staying the same – that is how you can tell that a selection is working. Each round they make the selection more stringent. (compound is omitted, incubation time is shortened, expression level of mutant enzymes is decreased.)
4. Get a student to explain the selection.
5. What are the results? Five clones are picked that have high activity for 2-oxovaline – each mutant had 23 to 28 point mutations evenly distributed. How good is the selection? How related are the five mutants? Mutants were as much as 10 to the 5th better than wt. One mutant preferred to use tyrosine as the aminotransfer AA.
6. How did the authors determine the relative importance of each mutation? Back mutagenesis.
7. What's the point? Is this only an academic evolution? No, glu analogs are useful therapeutics for mental disorders – glutamic acid is a neurotransmitter in CNS of vertebrates. It has important functions in memory and learning cognition. This group is looking for glu analogs and does a key transformation – amination of ketoglutaric acids by BCAT.
8. Cephalosporin acylase paper – The authors are trying to turn a glutaryl acylase into an adipyl acylase. Both enzymes are hydrolytic with different specificity for chain lengths. Look at picture from Nat biotech paper.

9. Authors divide the b-region of the gene into five parts and use EP-PCR to mutagenize each. Why do this? Why not just mutagenize the entire subunit at once? Too big! Is that too compromising for activity? Perhaps they wanted to understand the contributions of each individual mutation to activity better. What is their reported mutational frequency? Why might it be different for different parts of the gene?
10. The selection method requires making a non-antibiotic compound with a leucine side chain substituting for the B-lactam portion – hard to select with the real thing since it could kill all the clones. Kind of interesting, however, to run a parallel selection for an enzyme capable of doing the same activity in a resistant strain. Using this leucine derived compound (adipyl-leucine) in a strain that is auxotrophic for leucine provides selection for hydrolysis. The bacteria must hydrolyze the compound to live. This seems to be a special case – it wouldn't always correlate that hydrolysis of a distantly related compound translates to increased hydrolysis of the real thing – lucky break for the authors. The authors watched colonies appear over time – is this OK? Also, may be missing mutants that can do the real transformation.
11. How do the authors assess activity of their selected mutants? What is the fluorescamine assay? Draw mechanism and ask about sensitivity. Look at products being detected. Is this a reasonable assay? You can't test for product selectivity in a single assay – must assess preference for each compound separately.
12. Results – most mutations occurred early in the gene – 29 unique transformants. What is the AD/GL ratio? Is this a good way to evaluate mutants? They determine which single mutants have to most effect. Not a very good selection – not many rounds, so not much good stuff comes out of it. Asn-266 and Phe-375 – one has been previously described. Asn to serine mutation causes H-bond changes.
13. Problems – library generation. They would have been better suited to conduct shuffling or some other method to maintain the transformation efficiency of all library members. They had a lot of WT contamination leading to only five clones being selected. They don't do iterative library generation and selection as previous papers have done. Seems like they could have gotten just as good a mutant by doing iterative single site mutagenesis of reported important residues, as they suggest themselves.