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7.344 Directed Evolution: Engineering Biocatalysts □ □
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Session 4 Lecture Notes

1. How does chemical mutagenesis occur? Take a look at the chemdraw of the alkylation (explain term) of amine group-containing bases and how this causes AT to GC and GC to AT transitions (S_N2 -type displacement). Treatment of DNA with chemicals can result in purposeful incorporation of mutations. What are some other ways to chemically mutate DNA other than alkylation? UV light – thymine dimerization, nitrous acid – deamination of C, A, and G, formic acid depurination of DNA by breaking N-glycosyl bonds of purine bases, hydrazine deconstruction of pyrimidine rings.
2. What are the authors using this method to study? Proteolysis of fibrin using fibrinolytic enzyme. Fibrin is a protein involved in the clotting of blood, so this class of enzymes could help thrombosis. The authors tried to improve its activity in vitro.
3. What are the results? The specific activity of an identified mutant (h-mBsFE) is almost 100% increased over wild-type. 80% of the clones showed at least a 10% change in their activity – for better or worse. Mutations are occurring at 5 to 6 mutations per kb (0.6% - comparable to EP-PCR). Look at plate assay – does everyone understand how this was done?
4. What are the benefits to using this method? Fast, cheap, mutations are randomly distributed.
5. What are the pitfalls? Must use mutagens/teratogens for this procedure. This paper needs a selection to examine more library members. Seems like there is mutational bias even though the authors argue against it. Best mutant has only 1 AA change. Seems like a lot of work for a mutant that could be achieved with site-directed mutagenesis.
6. How could we make this strategy better? Combine with a recombination strategy following chemical mutagenesis.
7. Mutations using an e.coli mutator strain: What is the technique? How is it accomplished? A region of an antibody is cloned out and expressed in a mutator strain of e.coli (mutd5-FIT is error prone and XL1-RED is repair deficient) – can either be prone to single base substitutions or deficient in proofreading. Several round of mutagenesis are conducted (5) – bacteria are grown up and then diluted. Phage are then rescued by addition of a VCSM13 helper phage – meaning the phage origin of replication is now turned on and phage are produced. Phage have ScFV as a pIII fusion – externally expressed. Phage are panned against the antigen of the antibody for affinity (5 rounds). Phage recovered in final panning are then used to infect e.coli for production of the mutant ScFVs and examined for levels of expression and Ab affinity

using ELISA – ask if someone can explain how this is done. Several clones were expressed more efficiently – see western blot figure 5.

8. Authors are trying to use this technique to build better antibodies – specifically against HepB. Neutralizing Abs, vaccine development.
9. Calculating the mutational frequency: Total length of the scFv is ~717 bp (351+45+321). Divide this by the average number of mutations per retrieved clone – say this is a representative set of the total library (3.86 mutations per clone). Divide that by the total number of bp and you get roughly 0.5% mutation rate. How does this compare with other techniques?
10. What are the pitfalls to this method?
 - a. One must grow many rounds of bacterial cultures before mutational maturity is achieved. Each round takes about 12h, so very slow!
 - b. Low mutational frequency – especially for the work involved. Strong bias to transversion.
 - c. Because e.coli is being used as the mutator strain, there is a bias for codons that are e.coli compatible. All organisms have preferred codon usage (degeneracy of the triplet code for AA) and this isn't a problem if you are looking at prokaryotic proteins, but may be with eukaryotic proteins – in terms of expression (efficiency of AA incorporation.)
 - d. Finally, they didn't find and better clones in terms of affinity, only expression. This highlights the needle in the haystack problem – what if there is a high affinity clone, but very little is produced. Is the selection stringent enough to recover it?
11. For next week: genetic complementation. Show slide with picture of scheme. What is an auxotroph? In [genetics](#), a [strain](#) is said to be auxotrophic if it carries a [mutation](#) that renders it unable to synthesize an essential compound.
12. Aspartate 2-oxoglutarate aminotransferase activity – explain it. Slide of reaction
13. Discuss cephalosporin biosynthesis and customization.