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7.344 Directed Evolution: Engineering Biocatalysts □ □  
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## Session 11 Lecture Notes

1. The authors' primarily address transformation efficiencies as limiting steps. Cytotoxicity is mentioned as well as the expression hosts' ability to escape selection pressure by increased protein expression or alternative metabolism. If your downstream application is in vivo these points are useless.
2. Ribosome display is the strategy. See Figure 1. The authors use an enrichment for activity based on the binding of active site inhibitors – see figure 2 for a mechanistic explanation. A linker protein is fused to the enzyme to allow for proper protein folding and reactivity. Biotin is tethered to the inhibitor allowing for pulldown of active complexes. Dissociation of the complex with RNase and sequencing (RT PCR) is used to identify useful mutants.
3. Library size is only limited by the total number of active ribosomes in the reaction mixture – differs depending on the IVT kit.
4. The authors do many controls. First they test their tethered substrate. Would be nice to see the kinetic data that they don't show on the same curve for comparison's sake. They QC for ribosome-bound reactivity by checking to see if activity levels are comparable after RNase treatment. Why do they see only 50% of the total lactamase in complex? Is proteolysis really a possibility? How else could enzyme be released? What if mutagenesis led to stop codon incorporation? Also, walk through figure 6. (Controls for the enrichment)
5. The authors see a 100+ fold enrichment for a single round of selection using a mock selection with 1:10 mixed active to inactive enzyme. Would be nice to see a greater ratio of inactive clones.
6. Problems: Still requires a chemical tethering strategy. Non-specific labeling of the substrate to the bound enzyme seems to be a problem. Dissociation of the enzyme from the ribosome – loss of genotype/phenotype link. Seems like a very fast protocol, however. Cross reactivity? Probably concentration dependent – also linker dependent. You want to use the shortest possible linker for each system.
7. The strategy requires DNA hybridization of a ds fragment to the mRNA and then ligation of another ds DNA fragment that is biotinylated.
8. Confirmation of the mRNA/dsDNA complexation was performed using a shift assay. Hybridization was equally efficient for all linker lengths. Efficiency is at about 50% (not very good – losing half of you library right here!) Lots of controls were run for linker length. It turns out to be very

important in this case – efficient ligation was only observed with the longest linker.

9. The authors do a 1:1, 1:10 and 1:100 mix with inactive mutant mRNA. Selection is efficient and tops out around 40 fold. Does figure 5 show the results one would expect? Why does the enrichment increase with decreasing amounts of active mRNA?
10. Biggest two problems: cross reactivity and poor dsDNA hybridization.
11. Only one example of evolution: dihydrofolate reductase using immobilized methotrexate on agarose beads (substrate analog method).