426b Pooling of Enzyme Libraries for High Throughput Biocatalyst Development

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Biocatalysis is becoming increasingly important to the synthesis of pharmaceutical intermediates, aided by the ability to use directed evolution to tailor the specificity and stability of the biocatalyst for bioprocess optimization. Once a directed evolution library of enzymes is generated, a significant amount of effort is expended in high throughput screening to identify the improved mutants. Unfortunately, combinatorial bioengineering methods produce far more variants than most laboratories have the capacity to screen. Thus, the discovery of the best mutants becomes driven by the probability of sampling these from the screening pool. Pooling multiple mutants into each assay well increases the accessible sequence space, but must be balanced by the detection limit of the assay. We have mapped out the conditions for effective pooling, particularly with regard to assay accuracy, the fraction of good mutants created, and their activity level improvement. First, we developed Monte-Carlo simulation models of pooling and experimentally validated them using progressively complex scenarios, ranging from mixing just a rarely occurring “supermutant” in low frequency with an ancestral enzyme to the screening of an actual directed evolution library. In each case, the number of “supermutants” detected by the simulation model and in the experiment was nearly identical. Pooling increases the chances of detecting the "supermutant" in all cases, regardless of assay accuracy. Subsequently, using pooling to evolve β-galactosidase into a β-fucosidase, we verified the effectiveness of pooling over a conventional evolution approach.