530e Generating Libraries for Directed Evolution of Proteins – Comparison of Recombination-Dependant Pcr and DNA Shuffling

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Good library creation is the most important step in successful directed evolution experiments. The screening of suboptimal libraries that contain a high background of parental templates is a waste of time and resources.

In this investigation, we have compared systematically the utility of DNA-shuffling protocols with recombination-based PCR protocols on b-lactamase and fluorescent proteins. DNA-shuffling is a commonly used method to generate libraries, but is disadvantaged by the presence of a high parental background. We used variations of recombination-based PCR protocols which necessitates at least 1 crossover for amplification.

Working towards the goal of evaluating the recombination protocols for different recombination needs, we created 3 scenarios of gene recombination. In the first case, recombination of point mutations were done on b-lactamase gene using DNA-shuffling and recombination-based PCR protocols. In the second scenario, we recombined highly homologous genes of >70% from fluorescent proteins. Lastly, we explored the limits of the lowest homology we can recombine with the current recombination protocols. The findings provide a guide for more efficient and successful gene recombination.