INSILICO GUIDED CRISPR-CAS DRIVEN ENZYME ENGINEERING FRAMEWORK: AN AUTOMATED AND EFFICIENT ENZYME ENGINEERING METHOD

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Enzyme engineering is a time and resource-consuming process. *Insilico* methods specifically, the Artificial intelligence-based screening process accelerates enzyme engineering and CRISPR-CAS technology has revolutionized gene editing. Combining these two powerful methods, we have developed an automated and efficient enzyme engineering process. Initially, *insilico* studies will be used to derive specific point mutations or a region that can be randomly mutated. These are incorporated into the gene-editing technique CRISPR-Cas for any gene of interest such as the enzyme (Ex: Transaminases). The gene of interest will be on a plasmid within a bacterial cell and the mutations will be incorporated into the gene of interest using CRISPR-Cas technology. CRISPR-Cas will be used for introducing mutations that are both specific as well as random. The clone with different combinations of mutation is screened for enzyme activity using a zymogram based assay or a colorimetric assay to qualify the clones or enzyme. The qualified enzymes are sequenced and the sequence with the activity data is fed into an AI tool for further design and Crispr cycle. This cycle is continued till qualified enzymes show a considerable color change suggesting high activity. We are presently engineering a transaminase enzyme using this technology with 50 cycles. This presentation will include the technical details and results obtained so far from engineering different enzymes using this technique.

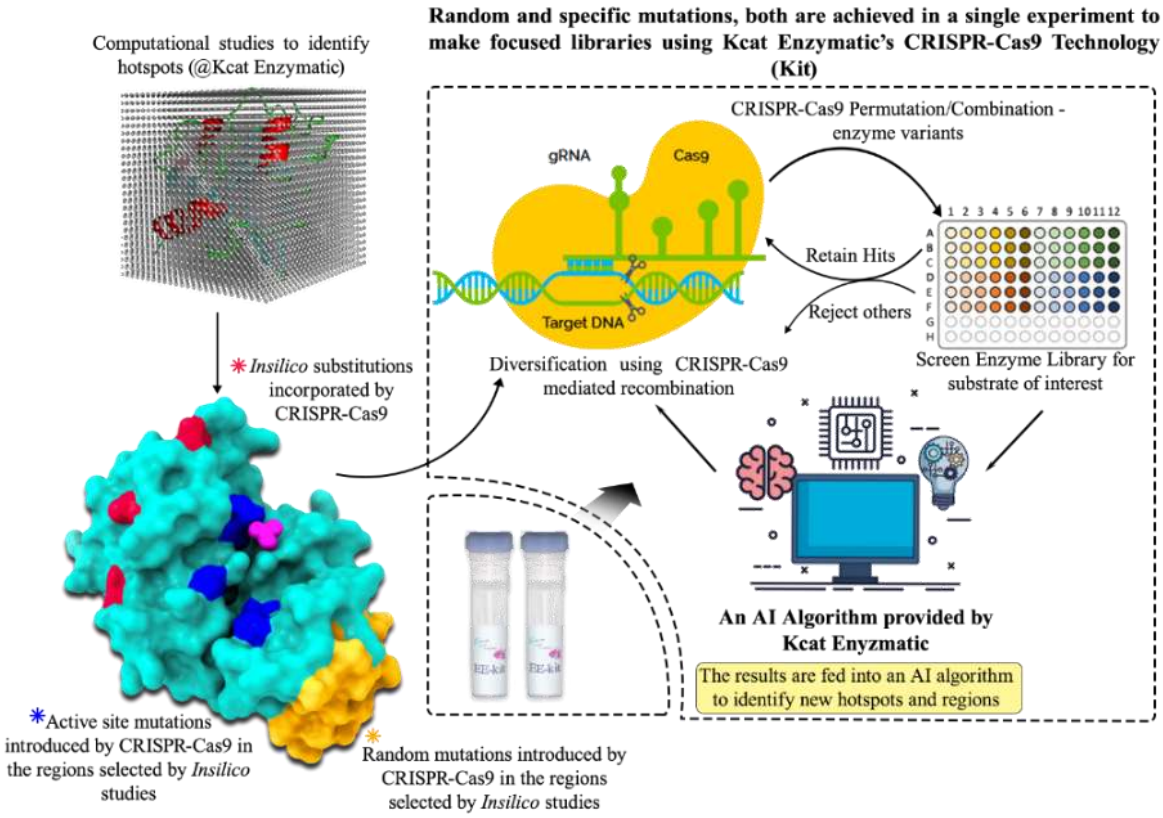

Figure 1: is a schematic showing the whole process from designing the mutations, conducting the CRISPR-Cas experiments, and selecting the enzyme clone with the best activity to be fed back into the cycle.