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Biofoundry based Automated Mutant Cloning Workflows

Jihyeon You[1,2], Aporva Gupta[3], Seong-Kun Bak[1,2], Wonjae Seong[2], Dae-Hee Lee[1,2,3],

Seung-Goo Lee[1,2,3], and Haseong Kim[1,2,3*]

[1]*Synthetic Biology and Bioengineering Research Center, Korea Research Institute of Bioscience and Biotechnology (KRIBB), Daejeon 34141, Republic of Korea*

[2]*Graduate School of Engineering Biology, Korea Advanced Institute of Science & Technology (KAIST), Daejeon 34141, Republic of Korea*

[3]*Department of Biosystems and Bioengineering, KRIBB School of Biotechnology, University of Science and Technology (UST), Daejeon 34113, Republic of Korea*

**Corresponding author: haseong@kribb.re.kr*

Protein engineering is critical in optimizing industrial enzymes for enhanced performance. While AI-based in silico mutant libraries and activity prediction models are commonly employed, experimental validation remains crucial. Researchers face significant challenges in generating protein mutants, often requiring extensive time and labor. To address these issues, we have developed workflows for protein mutation generation using automated machines. We efficiently generate mutations through site-directed mutagenesis and directly introducing the mutant DNA mixture to competent cells using a series of customized steps. The clones were verified via high-throughput nanopore sequencing, enabling to confirm the genotypes of multiple samples in a single run. As a result, we were able to generate 96 variants with different site mutations in approximately 4 days. The proposed method generating a large-scale dataset will play a significant role in speed up to understand the phenotypic landscape of a protein for further engineering and optimization of proteins.