Assembly of precise mutant libraries using arrayed-synthesized oligos for protein optimization

Application Note

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Assembly of precise mutant libraries using arrayed-synthesized oligos for protein optimization

Introduction

Protein engineering is a critical process in the development of protein and antibody-based therapeutics as well as in the optimization of industrial enzymes for chemical and agriculture purposes [1, 2, 3]. Two methods to improve protein characteristics that are employed are rational design and directed evolution **(Figure 1)**. Rational design involves precise changes in amino acid sequence with preconceived knowledge of protein structure and function and are typically introduced using site-directed mutagenesis [4]. Directed evolution, a method awarded the Nobel prize in chemistry in 2018, does not require knowing protein structure and function, but rather, uses randomization to create a library of mutant variants. Through high-throughput screening, mutants with improved properties are selected and can be further improved through multiple rounds of mutation and screening [5]. Critical factors that determine the outcome of directed evolution efforts are the availability of **high-quality mutant libraries** and **high-throughput screening methods** to assess large numbers of individual variants.

Traditional methods for library construction, such as using error prone polymerase chain reaction (PCR) or degenerate oligonucleotides, introduce mutations across the sequence space [6]. However, there is limited control over the codons introduced and variants incur significant codon bias. This results in a large library with poor variant representation, causing a large screening effort on the part of the researcher to ensure they are capturing the entire variation of the library.

Using GenScript's advanced arrayed, semiconductor-based oligonucleotide synthesis platform in mutant library construction allows precise control over mutation ratio resulting in a well-designed, diverse mutant library containing only the desired variants. This method employs a semi-rational design to improving protein characteristics, thus saving valuable time and effort during the screening process, speeding up discovery workflow, and reducing the overall cost of downstream expenses [4].

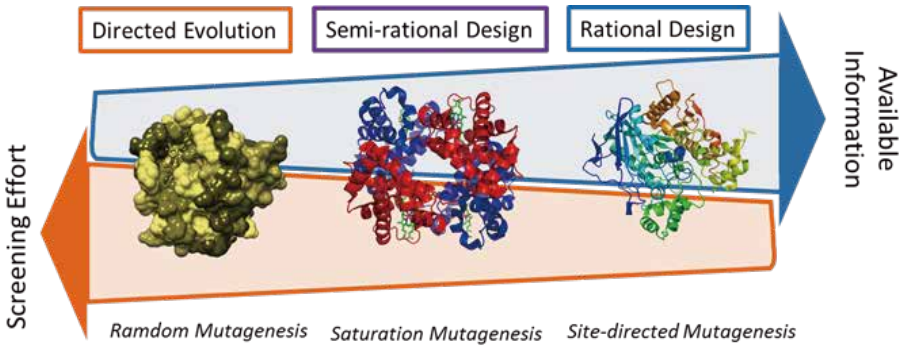

Figure 1: Semi-rational design combines benefits of directed evolution and rational design to improve protein characteristics. Multiple, specific residues are mutated based on prior structural and functional knowledge to create smarter libraries that reduce screening burden and lead to faster identification of improved proteins.

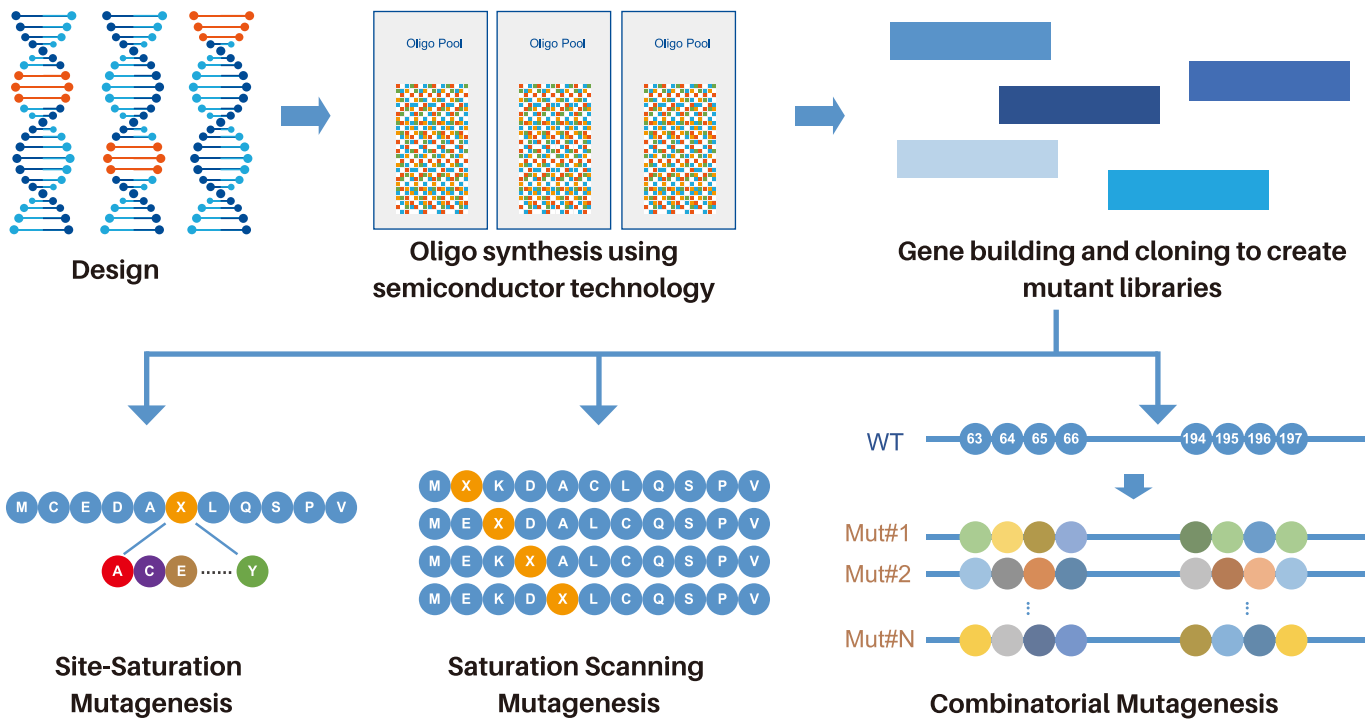

Figure 2: Workflow for constructing mutant libraries using arrayed, semiconductor-based oligos. Oligos are synthesized using our advanced semiconductor technology to create mutant variants and cloned into vectors to produce various types of mutant libraries. A site saturation or a saturation scanning mutagenesis library involves mutating positions located throughout a protein or within consecutive regions to all 20 amino acids or specified codons. A combinatorial mutagenesis library involves mutating multiple positions simultaneously across target regions of a protein, with each variant containing multiple mutations.

860 Centennial Ave., Piscataway, NJ 08854, USA
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In this Application Note, we provide two examples of how our array-synthesized oligos can be utilized in the construction of precise, well-designed mutant libraries for optimization of protein function. Synthesized oligos were used to create mutant variants that were cloned into vectors to create multiple types of mutant libraries **(Figure 2)**. Here, we show how the use of array-synthesized oligos can be utilized in the construction of a saturation scanning mutagenesis library and a combinatorial mutagenesis library.

Case Study #1

Saturation mutagenesis libraries involve systematically mutating every position in a specified region of interest or across an entire protein sequence space. At each mutated position, the wild-type amino acid coding sequence is replaced with codons for all 19 common, non-wild-type amino acids, with each variant in the library ending up containing a single mutation at one position within the protein. This type of library is great for functional characterization of a protein or optimization of the function of key regions within the protein sequence.

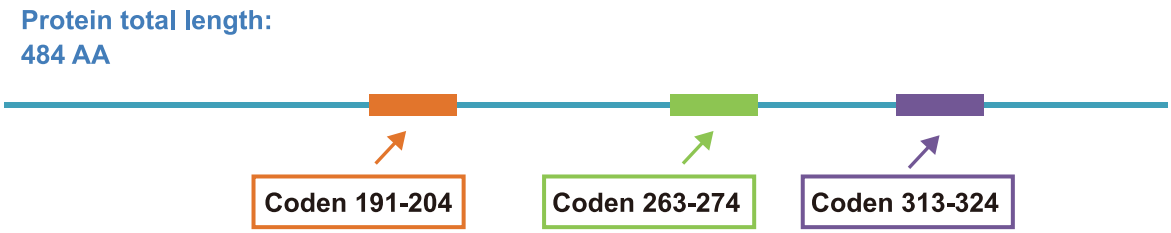

Figure 3: Design of a saturation scanning mutagenesis library of a 484 amino acid enzyme with three mutated regions of interest.

Equal representation of amino acids at each position

Library quality was analyzed using next-generation sequencing (NGS) to verify variant frequency. At each position, there was near 100% coverage of all desired mutated variants. In addition, there was relatively equal representation of amino acids per codon position, indicating that there was no over- or underrepresentation of variants in the library **(Figure 4)**. Therefore, utilizing arrayed, semiconductor-based oligo synthesis in mutant library construction provides precise control over the codons used in each variant. This ensures equal representation of amino acids at all mutated positions and, thus, avoiding poor representation of variants within the library.

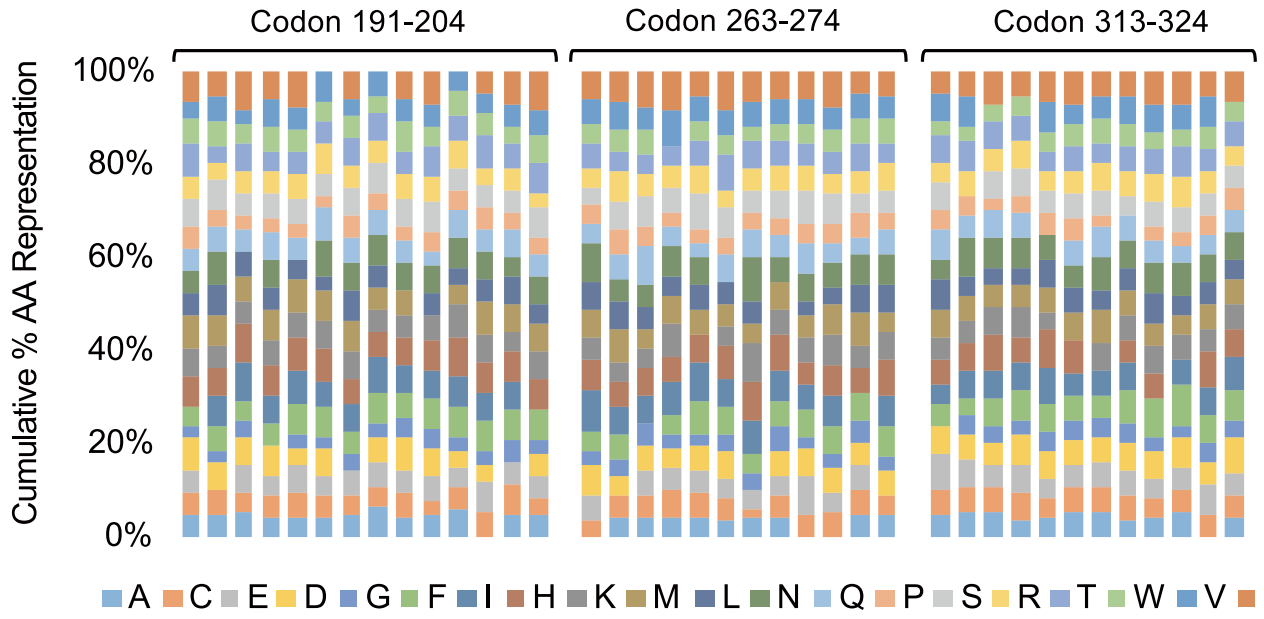

Figure 4: Equal representation of amino acids at each position. NGS verification of variant frequency was performed. Each bar represents a mutated position and each color represents the frequency of variants with a particular amino acid. Amino acids are represented by letter and color.

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Case Study #2

Combinatorial mutagenesis libraries involve mutating multiple positions simultaneously across target regions of a protein, with each variant containing multiple mutations. This type of library allows the researcher to fine tune a protein sequence with specific mutations and precisely control the number of mutations per variant. In addition, ratios of amino acids can be controlled providing a great tool, for example, to optimize antibody function by affinity maturation or precise engineering of antibody CDR regions.

A combinatorial mutagenesis library for two separate regions within the protein, each containing 4 sequential codon positions to be mutated was created. Desired amino acid distribution varied at each codon position within both regions. Each position was mutated simultaneously and were recombined to generate all combinations of desired variants **(Figure 5)**.

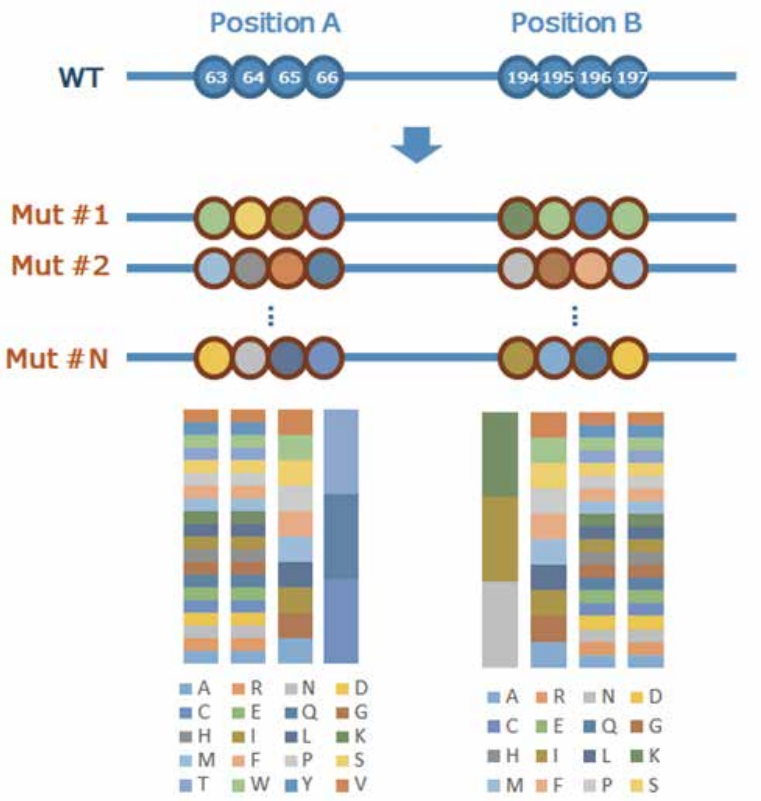

Figure 5: Design of a combinatorial mutagenesis library for 4 sequential codon positions in two separate regions of a protein.

Each region to be mutated within the protein required different amino acid ratios at each codon position. For example, for region B, the designed distribution of amino acids required variants to contain only 3 amino acids in the first position, 10 amino acids in the second position, and all 20 common amino acids to be present in the final two positions. Following library construction, amino acid distribution among the variants was determined using NGS. The actual distribution of the created mutant library matched the designed distribution for region B **(Figure 6)**. Similar data was obtained for region A. In addition, NGS confirmed the presence of less than 20% INDELs at mutated positions.

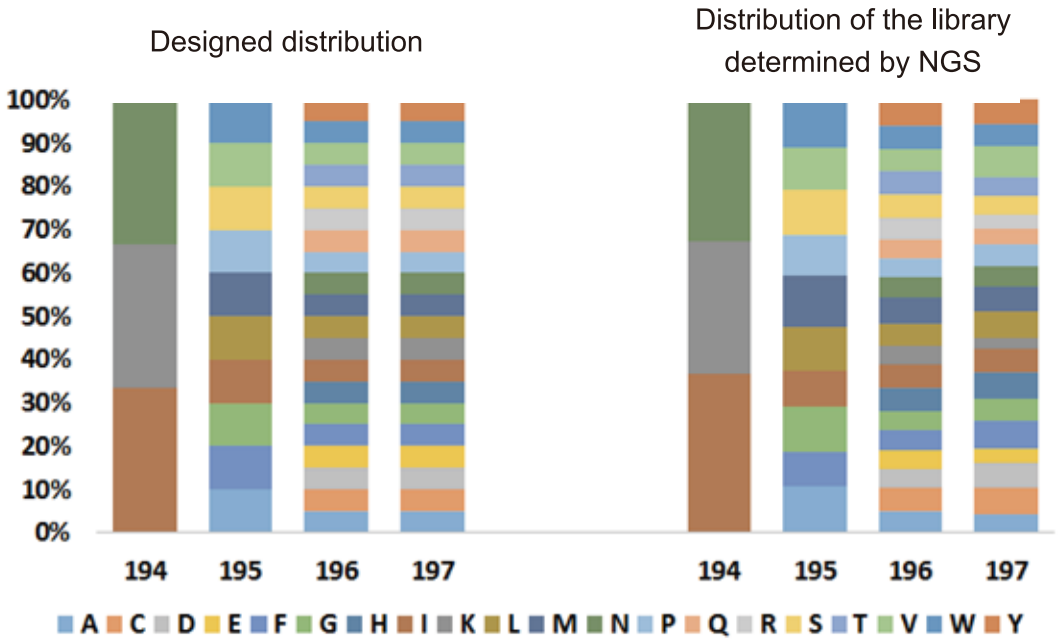

Figure 6: Distribution of amino acids within the variants of the combinatorial mutagenesis library matches the designed distribution. Each bar represents a mutated position and each color represents the frequency of variants with a particular amino acid. Amino acids are represented by letter and color.

Next generation semiconductor technology prevents codon bias in library construction

Traditionally degenerate oligonucleotides are used to introduce mutations across the sequence space. However, there is limited control over the codons introduced with degenerate oligos and variants incurring significant codon bias. In order to determine how using arrayed-synthesized oligos in library construction affects codon bias, NGS analysis of amino acid distribution was performed. Codon distribution was significantly biased for libraries constructed with NNK degenerate oligos, however, using our advanced arrayed, semiconductor-based oligo synthesis technology, we were able to achieve the designed amino acid distribution at each mutated position **(Figure 7)**. In addition, there was equal representation of variants containing the desired amino acids at each mutated position. Therefore, using semiconductor-based oligos in mutant library construction provides desired amino acid ratios at each mutated position and eliminates codon bias and poor representation of desired amino acids, thereby increasing screening efficiency and speeding up discovery workflow.

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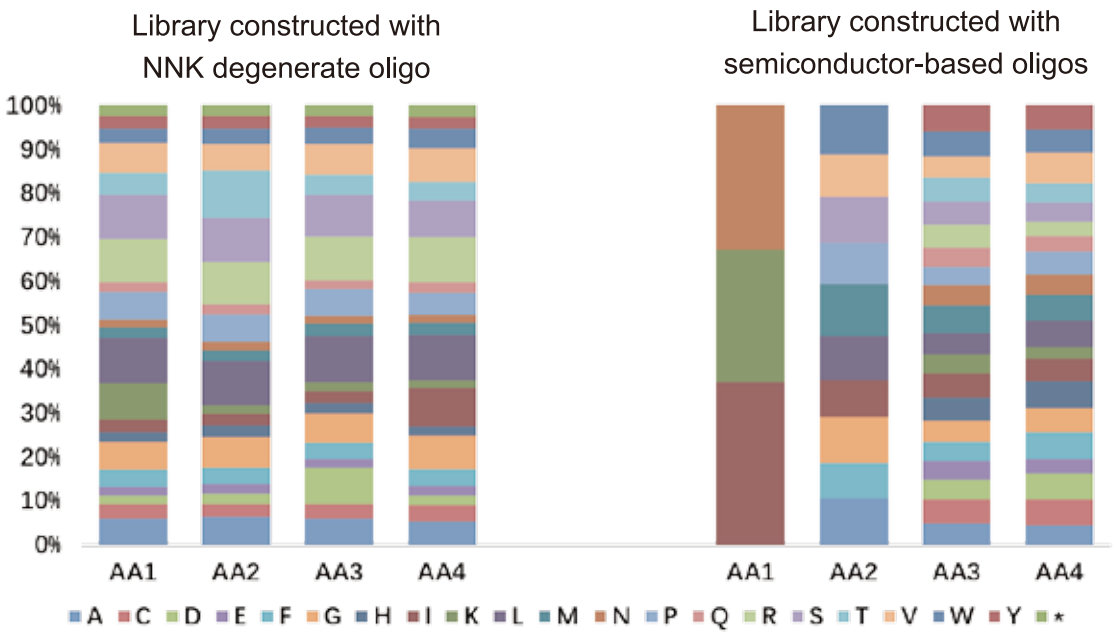


Figure 7: Comparison of amino acid distribution between libraries constructed using NNK degenerate oligos and those using our advanced arrayed, semiconductor-based oligo synthesis technology. Each bar represents a mutated position and each color represents the frequency of variants with a particular amino acid. Amino acids are represented by letter and color.

Summary

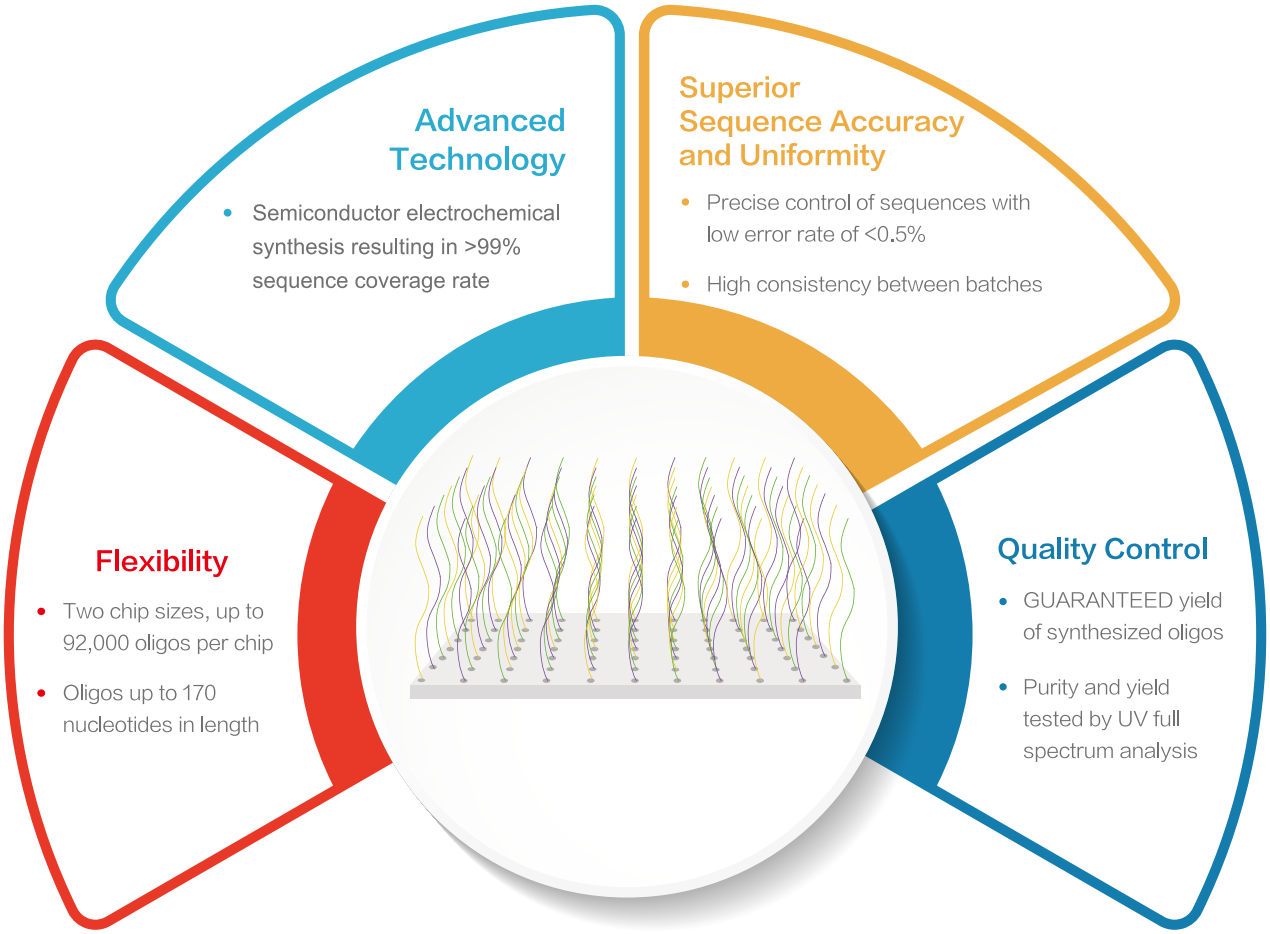
Mutant libraries can be used for the identification of critical residues within a protein or to optimize protein function, such as enhancing expression levels, modulating protein stability and activity, or increasing binding affinity with desired binding partners. Traditional methods for creating these libraries suffer from limited control over the codons introduced and variants can incur significant codon bias and early termination. This results in a large library with over- or underrepresented variants and incomplete variants, causing a large screening effort on the part of the researcher to ensure they are capturing the entire variation of the library.

GenScript has developed a method for creating diverse, well-designed mutant libraries using our advanced oligo synthesis platform. Our proprietary semiconductor-based electrochemical oligo synthesis technology allows customizable codon distribution at each mutated position, preventing the introduction of unwanted or stop codons and limiting codon frequency bias. Comparison of library distribution determined by NGS revealed codon distribution was significantly biased in libraries constructed with NNK degenerate oligos compared to the defined distribution achieved using arrayed, semiconductor-based oligos. Using arrayed-synthesized oligos provides precise control over the mutated codons used, creating a well-designed library that contains all the desired variants with unbiased distribution and thus minimizing the screening burden and saving the researcher valuable time and effort.

References

1. Engqvist, M.K.M. and K.S. Rabe. Applications of protein engineering and directed evolution in plant research. Plant Physiology, 179:907-917 (2019)
2. Nazif, A.M. et al. Maximizing stability in industrial enzymes: rational design approach. American Journal of Chemical Engineering, 5(6):135-139 (2017).
3. Tobin, P.H. et al. Protein engineering: a new frontier for biological therapeutics. Current Drug Metabolism, 15 (7):743-756 (2014).
4. Lutz, S. Beyond directed evolution – semi-rational protein engineering and design. Current Opinion in Biotechnology, 21(6):734-743 (2010).
5. Davis, A.M. et al. Directing evolution: the next revolution in drug discovery? Nature Reviews Drug Discovery, 16:681-698 (2017)
6. Bratulic, S. and A.H. Badran. Modern methods for laboratory diversification of biomolecules. Current Opinion in Chemical Biology, 41:50-60 (2017)

GenScript's Oligo Pool Service



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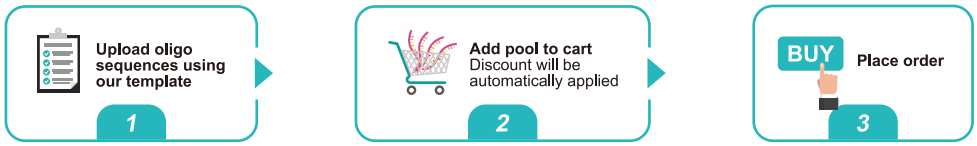

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