

P001 Using transposons to generate random amino acid deletions, insertions and substitutions in a target protein.

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Our ability to mutate genes *in vitro* has greatly enhanced our understanding of proteins and has allowed their adaptation for new applications. To facilitate such work, we have developed a series of simple methods that can remove, duplicate or substitute 3 bp at random positions in a gene, producing the deletion, insertion or substitution of a single amino acid within the protein. The method uses engineered transposons to act as the vector for inserting the recognition sequence for a type IIS restriction endonuclease. Upon removal of the transposon by endonuclease digestion, a single break is introduced into the gene. Depending on the position of the endonuclease recognition site within the transposon, self-ligation of the DNA results in the removal or duplication of 3 bp. Substitution is achieved by replacing the deleted 3 bp through inserting a second DNA sequence. We have demonstrated these methods on our model protein, TEM-1 β -lactamase. The method avoids any presumptions about residues considered essential for the protein. It also has advantages over traditional directed evolution technologies as amino acid insertions and deletions can be sampled, and, with respect to substitution, only a single amino acid is mutated (as opposed to multiple mutations, spread throughout the gene with differing effects on the protein) and all 20 amino acids can be sampled.