Washington University in St. Louis
Office of Technology Management

NEW METHOD TO PRODUCE A DNA MUTATION LIBRARY

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T-015343

Background: A DNA point mutation is a single nucleotide base substitution, insertion, or deletion in a gene sequence. Point mutations are responsible for several diseases including cystic fibrosis, sickle-cell disease, polycystic kidney disease, microcephaly, and Crohn's disease. The study on the effect of point mutations and development of therapeutics that target the associated diseases relies on access to DNA point mutation libraries. Currently, the construction of a DNA mutation library is costly and requires the purchase of up to thousands of oligonucleotides of specific sequences. Researchers at Washington University in St. Louis have developed a novel method for creating a DNA point mutation library, which could be used in protein structure-function studies, in vitro protein engineering, and greatly expedite the interpretation of human disease variants.

Technology Description: In a typical linear DNA amplification protocol a template sequence is used in conjunction with a primer sequence in the presence of all four native nucleosides (dATP, dGTP, dCTP, and dTTP) and a polymerase. In this new technology, the primer extension process is randomly terminated with the incorporation of a universal base. The use of specially modified primers enables easy retrieval of the terminated extended primers from the reaction mixture and their reactivation to continue the primer extension under typical conditions to yield full length primers. The original template is degraded and removed. A subsequent PCR amplification produces single point mutated DNA strands with A, G, T, or C incorporated opposite to the position of the universal base in the extended primer. Once more, the specially modified primers containing the universal base are easily removed from the PCR reaction and the PCR amplification is continued under typical conditions to produce an all native DNA point mutation library (allelic series). The success of this method has been illustrated with the creation of libraries containing nearly every single nucleotide mutation within the coding region of the beta-lactamase gene which confers resistance to ampicillin. A single kit containing all reagents and protocols enables in-house creation of a DNA mutation library thereby eliminating the costs of the purchase of up to thousands of oligonucleotides of specific sequences. Such a kit enables comprehensive screening in drug development against diseases caused by point mutations.

Key Advantages:

- Creates every point mutation possible in a specific DNA sequence
- Greater control by limiting the mutation to a single nucleotide
- Eliminates the purchase of up to thousands of oligonucleotides of specific sequences
- Useful in comprehensive screening in drug development

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