Transposon-mediated Rescue Cloning of Unidirectional Deletion Libraries From Either End of Any Target DNA

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We describe a simple method for the construction of unidirectional deletion libraries from either end of any target DNA. The system utilizes a transposon containing a conditional origin of replication (R6Kγori) and a selectable drug resistance marker. After transposition into the target DNA, the transposition reaction products are amplified in a PCR reaction using a primer from the distal end of the transposon and a second primer from the desired end of the target. The PCR reaction products are end-repaired, self-ligated, and transformed into the appropriate bacterial host to generate the deletion library. It should be noted that the target DNA can be almost any DNA containing the desired sequence: an existing plasmid, cosmid, or BAC clone, a restriction fragment, or a PCR product from genomic DNA or any other source.

By combining the above approach at the DNA level with the use of a specialized expression transposon carrying a T7 promoter and RBS, it is possible to obtain unidirectional amino and carboxyl deletions at the protein level. These could be valuable for epitope mapping, domain mapping, and protein engineering.