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Cut-and-paste-based cloning strategy for large gene site-directed mutagenesis

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ABSTRACT. Site-directed mutagenesis is an essential technique for investigating the mechanisms of gene regulation on a molecular level, as well as for exploring post-translational modifications and functional structure at the protein level. Polymerase chain reaction combining *in vitro* synthesis of oligonucleotide primers allows for site-directed mutation to be performed with ease. However, site-directed mutagenesis is difficult when larger plasmids are involved. Here, we present a novel method for generating large gene site-directed mutagenesis products based on a cut-and-paste-based cloning strategy. This method uses 4 primers, incorporating relevant mutations and restriction enzyme site sequences, to generate 2 DNA fragments by polymerase chain reaction. The fragments are then ligated into TA cloning vectors. Large genes containing mutations of interest were obtained by cutting and then pasting, and then inserting one fragment into another T-vector. We demonstrated the practicality of this method by creating a G59S mutation within the p150^{Glued}-encoding gene.

Key words: Cut-and-paste-based cloning strategy; p150^{Glued}; Polymerase chain reaction; Site-directed mutagenesis

