Genetics and Molecular Research

Restriction-ligation-free (RLF) cloning: a high-throughput cloning method by *in vivo* homologous recombination of PCR products

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Genet. Mol. Res. 14 (4): 12306-12315 (2015)
Received May 25, 2015
Accepted August 6, 2015
Published October 9, 2015
DOI http://dx.doi.org/10.4238/2015.October.9.19

ABSTRACT. In this study, we optimized a restriction-ligation-free (RLF) method to save time and cost of constructing multiple plasmids with the same gene insert, and examined the efficacy of RLF on high-throughput multi-plasmid cloning. This method utilizes the precise DNA repair and recombination systems within *Escherichia coli*, which allows to bypass the *in vitro* restriction and ligation enzyme reactions commonly included in

routine cloning procedures. A homologous arm is linked to the 5'-end of the forward primer used to amplify both the target gene and vector. A different homologous arm is linked to the 5'-end of the reverse primer. Therefore, genes can be cloned into the vectors by homologous recombination after co-transformation of the amplified target gene and the linearized vector, which bear the same homologous arm on either end. More than twenty-four different plasmids were generated by this method, which uses two simple polymerase chain reaction steps. This method is highly efficient in cloning any gene of interest into any vector at any site without sequence constraints, as no restriction and ligation reactions are required.

Key words: *In vivo* cloning; PCR; High-throughput; Homologous recombination

