High-fidelity T-vector system with the minimum background for the efficient expression in *Escherichia coli*

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The TA-cloning greatly simplifies the overall cloning procedures by eliminating intermediate steps involved in endonuclease treatment and purification. Despite its usefulness in molecular cloning, however, the expression vectors hardly adopt the TA strategy to place an ORF of interest downstream of their regions because a significantly high population of background are generated resulting from self-ligation without the lacZ color screening which is not applicable to the expression platforms. In this study, we developed a novel technique using a specially designed DNA cassette containing ORFs encoding EGFP and CAT to overcome the limitations, and to enable any expression vectors to be converted to expressional T-vectors without damaging pre-designed expression platforms resident in native vectors. By the several modifications of vectors, the T-vector could be designed to show that only vectors harboring forwardly-oriented ORFs were selectively remained in chloramphenicol-containing medium. The population study to test the background level of the recombination illustrated that high-fidelity T-vector system developed in this study is a useful method for the various applications in genetic engineering or synthetic biology.