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A Golden Gate and Gateway double-compatible vector system for high throughput functional analysis of genes

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Highlights

- pGate vectors combine both advantages of the Golden Gate and Gateway cloning features.
- pGate vectors contain “Bsa1 and Bbs1” sites and “attL1 and attL2” sequences.
- pGate vectors function efficiently in various molecular biology experiments.

Abstract

A major research topic nowadays is to study and understand the functions of the increasing number of predicted genes that have been discovered through the complete genome sequencing of many plant species. With the aim of developing tools for rapid and convenient gene function analysis, we have developed a set of “pGate” vectors based on the principle of Golden gate and Gateway cloning approaches. These vectors combine the positive aspects of both Golden gate and Gateway cloning strategies. pGate vectors can not only be used as Golden gate recipient vectors to assemble multiple DNA fragments in a pre-defined order, but they can also work as an entry vector to transfer the assembled DNA fragment(s) to a large number of already-existing, functionally diverse, Gateway compatible destination vectors without adding additional nucleotides during cloning. We show the pGate vectors are effective and convenient in several major aspects of gene function analyses, including BiFC (Bimolecular fluorescence complementation) to analyze protein-protein interaction, amiRNA (artificial microRNA) candidate screening and as assembly of CRISPR/Cas9 (Clustered regularly interspaced short palindromic repeats, CRISPR-associated protein-9 nuclease) system elements together for genome editing. The pGate system is a practical and flexible tool which can facilitate plant gene function research.

Key words: Golden Gate assembly, Gateway cloning, BiFC, artificial microRNA, CRISPR/Cas9

1. Introduction

In recent years, gene function analysis has progressed rapidly with the accomplishment of genomic sequencing of many plant species[1]. An efficient and convenient gene cloning strategy can greatly facilitate this progress. With conventional cloning strategies, the DNA fragments are excised by type II restriction endonucleases to create sticky or blunt ends, then reassembled by T4 DNA ligase in a separate reaction[2]. Although this restriction endonucleases plus T4 DNA ligases method is easy to use, and still