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A Useful MCR Modification for Plasmid Vector PCR Cloning

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Abstract

There are several vectors available for a variety of research, and new modified plasmids are created yearly. Depending on how specific the studies were, the majority of them had various Multiple Cloning Regions (MCRs). The main issue with this trial-and-error approach is that many MCRs only contain a few number of Restriction Sites (RS), which frequently makes the subcloning procedure challenging.The MCR for a GeneClip vector that can be used to clone PCR products and move the insert from one plasmid to another has been carefully designed here. Therefore, with this handbook, anyone may now create MCRs for their own vectors.

Keywords : PCR Products Cloning, T-Vector, Subcloning, Multiple Cloning Region (MCR), Restriction Sites (RS)

INTRODUCTION

A single DNA (or RNA) segment can be amplified across many orders of magnitude using the polymerase chain reaction (PCR) method, producing a large number of copies of a specific DNA sequence. Since the discovery of the polymerase chain reaction [1], new modified PCR applications have emerged to carry out a variety of genetic modifications [2, 3, 4].

Depending on the goal of the experiment, several DNA polymerases might be utilised to improve DNA amplification. The most popular methods for amplifying DNA fragments are thermostable DNA polymerases like Taq, Tth, and Tfl. These polymerases can add a single base extension of deoxyadenosine to the 3'-ends of amplified products but lack proofreading activity. In order to circumvent restriction and ligation, the PCR products can be immediately cloned into linearized T-vectors thanks to 3'-Ts overhanging on DNA duplexes corresponding to their adenines [5, 6]. By incubating the PCR fragment with dATP and a nonproofreading DNA polymerase, 3'-overhangs can be added to blunt-ended products created by PCR with proofreading Pfu and Tli polymerases [7, 8, 9].

There are numerous T-vector methods available for effective PCR product cloning. Such T-vectors have MCRs surrounding their T-ends that contain RE sites suitable for insert transference. It can be challenging to choose an appropriate location for subcloning the insert from a subsidiary vector to the destination one because sets of RS are frequently constrained. Even though subcloning is a fundamental molecular biology technique, it takes a lot of time and is not very useful when an experiment needs many repeats, variants, or confirmations with various inserts or other objects. By creating a universal MCR that can be used for both cloning PCR products and transferring the insert from one plasmid, we attempted to get over these challenges.

Materials and Methods

Our experiment's major goal was to create double-stranded oligonucleotides as polylinkers with two XcmI recognition sites.versions of the GeneClip vector's location. When the modified vector was digested with the endonuclease XcmI, the 3'-Ts overhanging on DNA duplexes complementary to the adenines of the PCR products resulted from the placement of the XcmI recognition sites. It enables the direct cloning of PCR results into linearized T-vector.

Using the trial-and-error method, RE sites for the MCR design were selected using the software SnapGene Viewer 2, 5 [10] and the online resource Enzyme Finder NEB [11]. Russian company Syntol Company created the oligonucleotides mcsT1 and mcsT2 in vitro (reference sequences are mentioned in the supplemental material). By incubating oligonucleotides in a restriction buffer (BSA-free) for 5 min at 950C, 15 min at 650 in a water bath, and then cooling to room temperature, equimolar levels of oligonucleotides were annealed,temperature. The obtained synthetic MCR was kept at -200C for no more than a month.

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