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Cloning of PCR products after defined cohesive termini are created with T4 DNA polymerase

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Amplification of DNA sequences using the polymerase chain reaction (PCR) has become a powerful experimental tool for molecular biologists (1, 2). The cloning of amplified products is usually achieved through either blunt end ligations, or the incorporation of restriction enzyme sites into the PCR primers. However, the former process is inefficient and the latter requires linkage of non-homologous sequence to the primers. Furthermore, restriction enzyme cleavage of sites at or near the termini of DNA primers is inefficient, and cleavage at internal sites in the amplified DNA is an added risk. I present a new approach for cloning PCR products which avoids most of these problems.

PCR amplification was performed with two primers, 24 and 20 bp long respectively, the 5′ ends of which were homologous to the cohesive termini created by AccI and XmaI (isoschizomers of SalI and SmaI, respectively). These termini contain the palindromes CG and CCGG, followed in these primers by the base A or T (Fig. 1). The aim was to usei the 3′ to 5′ exonuclease activity of T4 DNA polymerase to remove 3′ terminal sequences in the PCR products, thus recreating the cohesive AccI and XmaI termini (Fig. 1). Approximately 500 ng of PCR DNA product was purified from an agarose gel and treated with T4 DNA polymerase in the presence of dATP and dTTP (each at 0.1 mM). In this reaction, the 3′ to 5′ exonuclease of T4 DNA polymerase should be limited to removal of only bases G and C, creating the requisite recessed termini. After the T4 reaction, the enzyme was heat inactivated and unincorporated nucleotides were removed with Sephadex G50. The 500 ng of treated PCR DNA was then ligated to 100 ng of a non-dephosphorylated plasmid cleaved with AccI and XmaI (using SalI and SmaI sites, respectively, in the polylinker of pT7/T3α18 [Bethesda Research Laboratories]; polylinker stuffer fragment was removed by gel electrophoresis). Ligation was performed in 10 μl at room temperature, overnight; 30 μl of DH5α bacteria (competence: 10^6 colonies per μg supercoiled pUC19) were transformed with 0.5 μl of this ligation mixture. An estimated 2500 recombinants per μg input PCR DNA were obtained. The DNA sequences of cloning junctions were as predicted.

The above procedure has several advantages over existing methods for cloning PCR products. Firstly, rather than incorporating cleavable 5′ retriction enzyme sequences in the primers, this method requires only short 5′ extensions to incorporate cohesive termini. This reduces both the extent of non-homologous 5′ sequences, and the expense of longer oligonucleotide primers. Secondly, only one enzyme step need be used to give cohesive ends for efficient ligation; use of restriction enzymes can be avoided. Thirdly, directional cloning is readily achieved by incorporating a different cohesive 5′ sequence in each primer. One constraint in the procedure is that both cohesive termini must contain palindromes of only C/G's, or only A/T's. Finally, although A and T nucleotides are present directly 3′ of the cohesive ends in the primers described here, this is unlikely to be a strict necessity.

This procedure is thus straightforward and efficient, and should be of significant value to those wishing to clone PCR products.

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