A simple method for cloning blunt ended DNA fragments

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The cloning of blunt ended DNA fragments, particularly those generated by polymerase chain reaction (PCR), is often difficult due to the inefficient ligation of blunt ends by T_4 DNA ligase (1). We have developed a simple procedure for the efficient cloning of blunt ended DNA fragments, even when only limited quantities are available. The method involves the ligation of the DNA fragment with an oligonucleotide linker containing a restriction enzyme site, PCR amplification of the product using the same oligonucleotide as primer, followed by restriction endonuclease digestion to generate cohesive ends and ligation into a vector with compatible ends. We have used the following protocol to clone numerous different DNA fragments.

In order to generate blunt ends containing 5' phosphates, the DNA fragment (~5–500 ng) was incubated at 37°C for 1 hr with 5 units T_4 DNA polymerase (BRL) and 10 units T_4 polynucleotide kinase (BRL) in the presence of 1× Stratagene Universal Buffer supplemented with 0.2 mM dNTPs and 1 mM ATP. The enzymes were then inactivated by heating to 68°C for 10 min and non-phosphorylated *Bam*HI linkers (5'-CCGG-ATCCGG-3') (BRL) added to 5–10 μ M. One unit T_4 DNA ligase was added and the reaction incubated at 37°C for 1–2 hr. Only one linker molecule will be joined to each 5' terminus of the blunt ended DNA since self ligation of the linkers does not take place (because they are non-phosphorylated); thereby increasing the efficiency of ligation between the linkers and the DNA. The reaction was then diluted to a linker concentration of 1–2 μ M, dNTPs added to 0.2 mM, and PCR amplification carried out using Amplitaq (Perkin Elmer Cetus) or Replinaase (Dupont-NEN) in the buffer recommended by the supplier. The

reaction was carried out for 25 cycles of denaturation at 94°C for 1 min, annealing at 37°C for 1 min, and extensions at 60°C for 0.5 min and 72°C for 0.5 min. A final 10 min extension at 72°C was carried out before terminating the reaction. The unused linkers from the ligation reaction act as primers and amplify the DNA during PCR and thus increase the yield. The reaction was then extracted with phenol/chloroform and linkers separated by polyethylene glycol precipitation of the amplified DNA (2). The DNA fragment was resuspended in 10 mM Tris pH 8.0, 1 mM EDTA, digested with *Bam*HI and ligated into dephosphorylated *Bam*HI-digested pBluescript SK⁻ vector using standard techniques (1). While we have only used *Bam*HI linker, other oligonucleotides may be used, provided they are high enough in G+C content to anneal during the PCR.

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