Cloning PCR Products

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Introduction

Cloning PCR products into a stable vector is often desirable for subsequent analysis such as high quality DNA sequencing, hybridization studies, expression, modification or further subcloning. A wide variety of strategies have been employed and are available in commercially supplied kits (see Page 30 for a listing of manufacturers of PCR cloning kits or vectors). These approaches include simple but relatively inefficient blunt ligation schemes that rely on the 5' to 3' proofreading activities of some enzymes such as *Pfu* polymerase (Costa and Weiner 1994). These enzymes can be used to create blunt-ended PCR products or to remove the 3' overhang generated by *Taq* polymerase, creating "polished ends". A modification of this approach uses the restriction endonuclease *Srf 1* to cleave the vector DNA and then supplies *Srf 1* in the ligation buffer to provide a higher steady-state concentration of digested vector, which forces the reaction to proceed in the direction of ligation of the insert, as the ligated product is not digestible by *Srf 1* (Simcox 1992). Other recent innovations include the addition of 5' AATTC tails to PCR primers followed by amplification in a mix containing phosphorothioate dGTP. The PCR product is then digested with Exonuclease III, which removes bases from the 3' end of the strand until it encounters the protected G, leaving an AATT "sticky end", perfect for ligation into a vector's *Eco R1* site. Yet another method uses the Lambda phage recombination/integration system rather than ligation as

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a mechanism for cloning (reviewed in Landy 1989). In this approach, attB sites are incorporated into the PCR primers and attP sites are added to the vector. When the PCR product, vector, and Lambda phage integrase (*Int*) are combined, the PCR product is inserted into the vector in a site-specific manner. The same system can then be used with Lambda phage excisionase (*Xis*) to remove the insert for transfer to a different vector. Finally, restriction endonucleases, such as Eam 1104 I, which cuts at a defined distance from its recognition site (but not within its site) are used in PCR primers to allow specific "sticky ends" to be produced (Padgett and Sorge 1996). However, most of these approaches are either inefficient, or require the use of proprietary vectors, enzymes, or reagents and are often costly.

One approach lends itself well to duplication in the laboratory using commonly available reagents, and hence is extremely cost effective. This approach relies on the fact that *Taq* polymerase has a nontemplate-dependent activity which normally adds a single nucleotide (almost always A) to the 3' ends of all amplicons (Clark 1988). The efficiency of direct cloning of PCR products can be improved by this one base overhang, which facilitates ligation when the complementary T base is added to the cloning vector. This cloning strategy is called T/A PCR cloning. If *Vent* and *Pfu* polymerases are employed in the PCR reaction, there are no 3'A overhangs because of the 3'→5' exonuclease activity of these polymerases. The blunt-ended PCR products derived from these PCR reactions can be cloned into blunt-ended vectors. Alternatively, a 3' A-overhang can be added by incubation with *Taq* polymerase at the end of the PCR cycles.

There are many commercially available TA cloning kits, however, it is relatively simple and inexpensive to make your own T/A cloning vector. Generating a T/A cloning vector in your own laboratory may also give you the advantage of accelerating experimental progress. For example, if further study in a mammalian expression system is needed and you have an eukaryotic expression vector, you can find or make a blunt-end restriction site in the multiple cloning site. Using the following protocols, any vector can be made into a T/A cloning vector. After cloning your gene of interest, you can directly express your gene without having to clone into a T/A cloning vector and subsequently subclone it into an expression vector. Because T/A cloning is bidirectional, restriction digestion patterns or sequencing must be used to

check for insert orientation. Further in vitro and in vivo expression can then be carried out. The following is a basic protocol to generate a home-made T/A cloning vector.

Outline

The entire protocol of generating T/A cloning is outlined in Fig. 1.

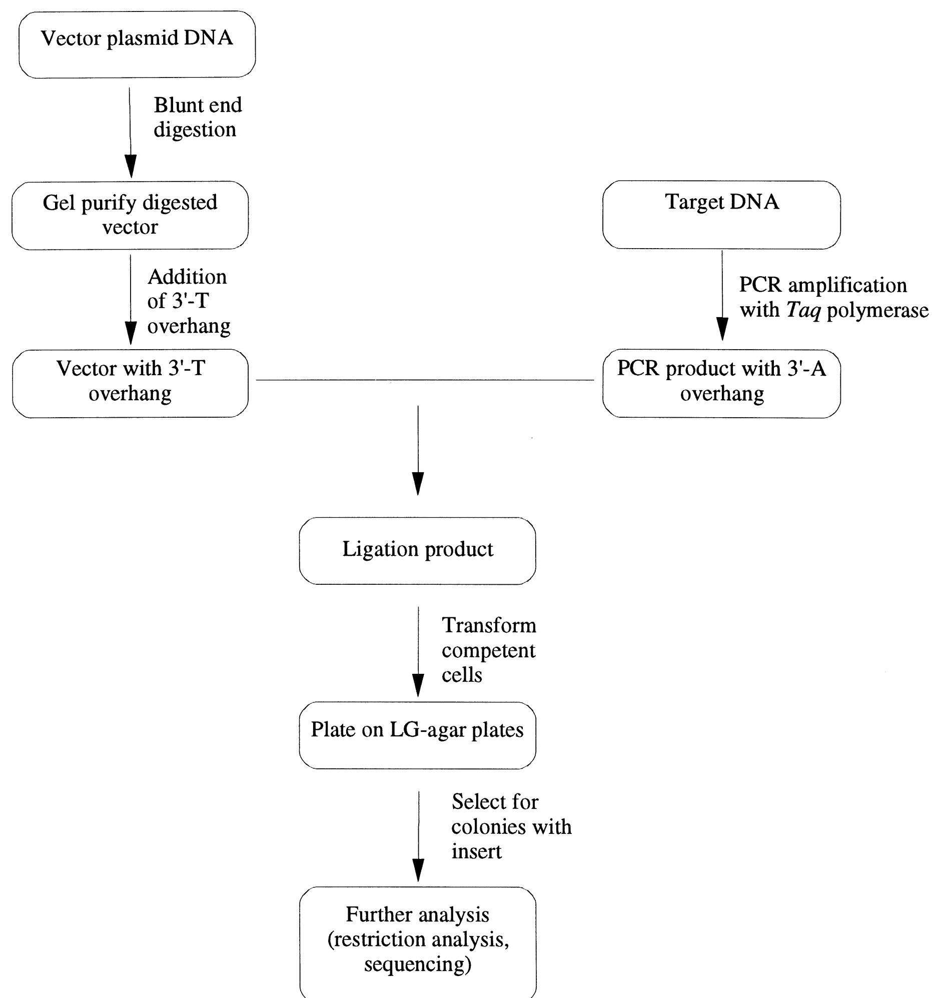

Fig. 1. Outline of T/A PCR cloning procedures