General method for direct cloning of DNA fragments generated by the polymerase chain reaction

David Kovalic, Jin-Hwan Kwak and Bernard Weisblum*

Pharmacology Department, University of Wisconsin Medical School, Madison, WI 53706, USA

Submitted May 21, 1991

Double-stranded DNA products obtained by primed amplification (polymerase chain reaction, PCR) with *Thermus aquaticus* (*Taq*) DNA polymerase contain a single 3' unpaired deoxyadenosine residue gratuitously added by the polymerase to its product (1). To clone the amplified products efficiently, the extra deoxyadenosine residues must either be removed or the vector must be modified to accommodate them (2, 3).

A general method is described for direct cloning of DNA fragments generated by primed enzymatic amplification that is based on digestibility of the cloning vector with *Xcm*I restriction endonuclease to yield a unit linear molecule with 3' unpaired deoxythymidine residues at both ends. Such vectors are capable of efficient ligation with products that are obtained by primed enzymatic amplification with *Taq* DNA polymerase bearing 3' unpaired deoxyadenosine residues at both ends. Other methods for direct cloning PCR products have been described which involve enzymatic tailing of the vector by addition of a single thymidylate residue with either terminal transferase and ddTTP (4), or, *Taq* polymerase and dTTP (5).

The restriction endonuclease preparation *Xcm*I (New England Biolabs, Beverly, MA) recognizes and cleaves the general class of DNA sequences (where N represents either A, T, C, or G), as shown below:

Two partially complementary 24-mer DNA oligonucleotides bearing the 5' *Nco*I overhang sequence, CATG, were therefore synthesized, annealed, and ligated with plasmid pGEM5fZ(+) (Promega, Madison, WI) that was previously prepared by digestion with *Nco*I at its single occurrence site in the multiple cloning sequence, as follows:

The resultant construction yielded, upon digestion with *Xcm*I, a vector with the desired unpaired 3' deoxythymidylate residues and central stuffer, as shown below:

This method has been used to clone directly the 213 nt *cop* (copy number control) gene sequence from staphylococcal plasmid pE194 (6), amplified by PCR. Ligation of PCR product with T-vector was performed in a total volume of 100 μ l that contained: *Xcm*-cut-, fractionated-, and electroeluted T-vector, 100 ng.; PCR-amplified *cop* gene sequence (213 nt long), 100 ng.; Tris HCl (pH 7.8), 50 mM; MgCl₂, 10 mM; ATP, 0.1 mM, and T4 DNA ligase (New England Biolabs), 1 Weiss unit. The reaction mixture was incubated at 16°C for ca. 16 hours. One μ l was used for electroporation of *E. coli* JM109 cells and yielded 240 ampicillin-resistant transformant colonies of which 178 (74%) were lac⁺, and 62 (26%) were lac⁻. DNA preparations from 9 randomly chosen lac⁻ colonies were analyzed. Seven clones contained insert monomers, 1 contained a dimer insert, and 1 contained no apparent insert. Four cloned *cop* PCR products were checked by DNA sequencing; the expected vector, vector-primer junction, primer, and insert sequences were found.

Mead *et al.* (7) have similarly designed T-vectors cleavable with either *Xcm*I or *Hph*I, both of which yield 3' thymidylate overhangs. They noted a variable loss of nucleotides from both the vector- and/or primer side (mostly 1 or 2 nucleotides) at the vector-primer junction, in products cloned using their *Xcm*I vector. In contrast, products cloned into their *Hph*I vector gave the expected sequence and showed no similar losses. The differences observed in the *Xcm*I vector preparations might be attributed to nuclease contamination of an early lot of *Xcm*I used by Mead *et al.* (7) which predated lot 4 that was used in our studies.

ACKNOWLEDGEMENTS

This work was supported by research grants AI-18283, S10-RR01684, and CA-07175 from the National Institutes of Health, DMB-8514305 from the National Science Foundation, as well as by research support from the University of Wisconsin Graduate School.

REFERENCES

1. Clark, J.M. (1988) *Nucl. Acids Res.* **16**, 9677-9686.
2. Jung, V. *et al.* (1990) *Nucl. Acids Res.* **18**, 6156.
3. Kaufman, D.L. and Evans, G.A. (1990) *BioTechniques* **9**, 304-306.
4. Holton, T.A. and Graham, M.W. (1991) *Nucl. Acids Res.* **19**, 1156.
5. Marchuk, D., *et al.* (1990) *Nucl. Acids Res.* **19**, 1154.
6. Byeon, W.-H. and Weisblum, B. (1990) *J. Bacteriol.* **172**, 7892-7900.
7. Mead, D.A., *et al.* (1991) *Bio/technology* **9**, 657-663.

* To whom correspondence should be addressed