Direct ligation of PCR products for cloning and sequencing

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Polymerase chain reaction (PCR) has made it possible to detect both the presence and expression of a gene within specific cell types using very small amounts of starting material. A number of modifications and adaptations of the PCR technique have been developed along with protocols for cloning the amplified products (1). These protocols, however, rely either on a restriction endonuclease recognition sequence built into the primers or take advantage of the template independent terminal transferase activity of Taq polymerase to add 'A' residues at the end of each molecule. Such molecules can then be ligated to a 'T' tailed vector. All these protocols require the purification of the PCR product in order to work efficiently. In our laboratory we are studying the expression of cytokines and T cell receptor variable region beta chain ($V\beta$) genes, using PCR, in arthritic joints of mice. To clone and sequence the PCR products rapidly we have developed a protocol which requires no manipulation such as gel purification, polishing the ends with T4 DNA polymerase and phosphorylation with T4 Polynucleotide kinase or use of special vectors. We have found that if the final extension step is omitted from the amplification protocol, the resultant DNA fragment can be ligated directly to M13mp18 vector digested with a restriction enzyme which generates blunt ends. We reasoned that since the PCR amplified material is basically a blunt end, dephosphorylated DNA molecule it can be ligated to a vector cut with either EcoRV or SmaI. The situation in this case would simply be reverse of that normal blunt end cloning where the vector is dephosphorylated while the termini of the target molecule carry the phosphate. In two separate experiments, using the same primers, a 330 bp segment of TCR $V\beta 6$ gene was amplified from genomic DNA. In the first experiment, the extension step was omitted from the amplification protocol while in the second experiment it was included. These two fragments were then subjected to ligation reaction using SmaI digested M13mp18 vector according to the following protocol. For cloning into T7 'Blue' vector (Novagen) which is a 'T' tailed vector, manufacturers instructions were followed.

Protocol. Digest 1 μ g of M13mp18 (RF form) DNA with SmaI at 30°C for 2 hrs. Check an aliquot on 1% agarose gel. If the digestion is complete increase the volume of the digestion reaction to 250 μ l with nuclease free water and extract twice with phenol:chloroform:isoamyl alcohol (2) and precipitate with 3 M sodium acetate, pH 6.2. Dissolve the pellet in nuclease free water

and adjust the concentration to 10 ng/ μ l. For ligation, to 2 μ l of linearized M13 DNA plus 4 μ l of 5 $\times$ Ligation buffer (BRL) are added different volumes of the PCR reaction, usually 1, 2, 4, 6 and 8 μ ls, in different tubes and the volume is brought up to 18 μ l. 2 μ l of T4 DNA ligase (BRL) is then added to the tube and mixtures are incubated at 16°C overnight. 1–2 μ l of this ligation mixture is used for the transformation of competent DH α F' cells (BRL) and plated according to manufacturers instructions. The plates are incubated overnight at 37°C and the positive plaques are either identified by Blue–White color selection or by screening with radiolabelled internal oligos. The results of one such screening are given in Table 1. The identity of the DNA fragment was confirmed by sequencing.

From the results shown in Table 1, it is apparent that high efficiency ligation of PCR amplified DNA fragments is possible without the use of special vectors and tedious protocols thus avoiding the expenses as well as saving time. Using this protocol we have successfully cloned and sequenced PCR amplified fragments ranging in size from 330 bp (TCR $V\beta 6$) to 640 bp (IL-6).

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REFERENCES

1. Innis, A.M., Gelfand, D.H., Sninsky, J.J. and White, T.J. (1990) *PCR Protocols: A guide to Methods and Applications*. Academic Press, Inc., San Diego.
2. Maniatis, T., Fritsch, E.F. and Sambrook, J. (1982) *Molecular cloning: A Laboratory Manual*. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.

Table 1.

Vector	# of plaques/ colonies	Extension Step	% Positive
M13mp18(Sma-1)	176	No	30
T7 'Blue'	127	No	2*
M13mp18(Sma-1)	147	Yes	0
T7 'Blue'	214	Yes	32

*Only two clones hybridized with the internal probe and both contained the correct fragment.