Ligation-independent cloning of PCR products with primers containing nonbase residues

Stefan Kaluz* and Anthony P.F.Flint
University of Nottingham, Sutton Bonington Campus, Loughborough LE12 5RD, UK

Received May 24, 1994; Revised and Accepted September 21, 1994

For many applications it is often necessary to subclone PCR products into plasmid vectors. Many strategies for subcloning PCR products have been developed but most require subsequent enzymatic treatment and/or purification. Ligation-independent cloning of PCR products (LIC-PCR) is a versatile method eliminating a time-consuming and occasionally problematic ligation step. In the method described by Aslanidis and de Jong (1) two amplifications are required — amplification of product with primers containing 12 nucleotide sequences lacking dCMP in the 5′ ends and amplification of a vector with primers containing 12 nucleotides complementary to product primers. Complementary single strands are produced by exonuclease activity of T4 DNA polymerase in the presence of dGTP and dCTP, respectively. Alternatively specific sequences devoid of particular bases can be incorporated into a vector, eliminating the vector PCR (2). Another method (3) also requires only amplification of product with primers containing 12 nucleotides complementary to any vector sequences flanking a restriction site. Complementary single strands are created by controlled Exo III digestion of product and linearized vector. In both cases annealed 12 nucleotide stretches are stable enough to transform competent cells. LIC-PCR kits based on method (2) are now commercially available (Clontech, Pharmingen).

An additional improvement to this methodology would be elimination of all enzymatic treatment of the PCR product and direct production of complementary single strands to predefined vector sequences during PCR. Abasic sites (nonbase residues) present in template DNA have been reported to markedly slow the incorporation of nucleotide triphosphates by DNA polymerases (4). Recently, a method using incorporation of nonbase residues into primers has been used to produce PCR products with single-stranded tails, nonbase residues serving as nonreplicable elements to terminate DNA synthesis. These modified primers were found to function as efficiently as 'normal' ones (5).

Here we report a rapid and simple method for LIC-PCR using primers containing the nonbase residue 1,3-propanediol in defined positions. Clonable complementary ends are produced directly in the PCR when Taq DNA polymerase stops at the nonreplicable element leaving the rest of the primer single stranded:

GCGGTGGCGGCXGCccagatgtgcatttaga..ttgtttaaaagttaaaGC
CGggtctactcgtaaatct..aacaaattttcaatttCGXCGGCGAGATCT

X = 1,3-propanediol, modified NotI recognition site is in bold. In this case, primers were designed to be complementary to the 12 nucleotide regions flanking the NotI site of the Bluescript vector. As 1,3-propanediol maintains the same atomic distance between the 5′ and 3′ carbon atoms as deoxyribose and allows complete freedom of rotation about the carbon–carbon bonds (5), this nonbase residue does not destabilize annealed complementary single strands. After transformation, nonbase residues are removed and corresponding vector nucleotides are copied by the repair mechanism of bacterial cells.

A short outline of the method is as follows. The PCR solution is extracted with phenol/chloroform and chloroform to inactivate Taq DNA polymerase and remove mineral oil. With no further treatment about 50 ng of PCR product is mixed with an equimolar amount of NotI linearized and Exo III digested (6°C, 1 min) Bluescript in 70 mM Tris–HCl, pH = 7.6 containing 10 mm $MgCl_2$. The mixture is heated to 70°C for 10 min and allowed to cool to ~30°C. An aliquot is used to transform competent cells. Using XL 1 Blue cells we found that in all recombinant clones tested the NotI site was restored as indicated by NotI digestion and sequencing through primer regions. The method was tested using three different sheep Type I interferon receptor genomic and cDNA PCR products with the following results (number of positive clones/number of clones tested): Product 1, 10/10; Product 2, 9/10; Product 3, 9/10. Experiments with primers containing a cytosine in place of the propanediol residue ('normal' primers) produced no recombinants confirming that single strands produced by termination at the propanediol residue are essential with total overlap between PCR product and vector as short as 22 bp. The overall efficiency of this method is somewhat lower than that of conventional methods being within the range of $5\times10^2-1\times10^3$ colonies per microgram of PCR product. To increase efficiency it might be interesting to test other non-base residues.

We have demonstrated applicability of nonbase residue 1,3-propanediol containing primers for LIC-PCR. The method described reduces the number of steps in directional cloning of PCR products. Unlike cloning in T-vectors the method does not require treatment of PCR products, while offering directional cloning without a ligation step.

REFERENCES

1. Aslanidis,C. and de Jong,P.J. (1990) *Nucleic Acids Res.* **18**, 6069–6074.
2. Kuijper,J.L. *et al.* (1992) *Gene* **112**, 147–155.
3. Hsiao,K.Ch. (1993) *Nucleic Acids Res.* **21**, 5528–5529.
4. Randall,S.K. *et al.* (1987) *J. Biol. Chem.* **262**, 6864–6870.
5. Gade,R., *et al.* (1993) *Genetic Analysis. Techniques and Applications* **10**, 61–65.

* To whom correspondence should be addressed