

Improved cloning efficiency of polymerase chain reaction (PCR) products after proteinase K digestion

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Submitted November 14, 1990

We and others have occasionally experienced difficulty in cloning PCR derived DNA fragments (1). This has occurred even after extraction of the DNA with phenol and phenol:chloroform, followed by precipitation with ethanol and/or column purification of the bands.

To test the hypothesis that the *Taq* polymerase remains bound to the DNA and therefore inhibits restriction endonuclease activity, we incorporated a proteinase K digestion step prior to endonuclease digestion and compared the frequency of cloning of the PCR derived products. We report here an example of the increased cloning efficiency we have achieved after the incorporation of the proteinase K digestion step.

PCR reactions were carried out using a human immunoglobulin K chain cDNA template. The reactions were performed in a programmable heating block (Hybaid) using 25 rounds of temperature cycling (94°C for 1 min, 50°C for 2 min and 72°C for 3 min) followed by a final 10 min step at 72°C. Eight hundred nanograms of each primer, 100 ng of template and 2.5 Units of *Taq* polymerase (Perkin Elmer Cetus) were used in a final volume of 100 μl with the reaction buffer as recommended by the manufacturer. Synthetic oligonucleotides were made on a 7500 DNA Synthesiser (Milligen), and were designed to amplify the 355 bp constant region of the K chain cDNA. The primer oligonucleotides were unphosphorylated and had a complementarity of 20 nucleotides with the template at the 3′ end, a *Hin*dIII site 5′ to this and a 4 base extension 5′ of the *Hin*dIII site. On completion of the reaction the PCR product was divided into 2 equal parts. The non-proteinase K control was subjected to phenol:chloroform and chloroform extraction follwed by ethanol precipitation. The proteinase K fraction also received phenol:chloroform and chloroform extractions and was subsequently adjusted to 5 mM EDTA, 10 mM Tris (pH 8.0), 0.5% SDS. Proteinase K was added to a final concentration of 50 μg/ml and incubated for 30 minutes at 37°C and then 68°C for 10 minutes. The reaction mix was then extracted once with phenol:chloroform and once with chloroform before ethanol precipitation. The non-proteinase K and proteinase K reactions were subsequently treated identically and were digested with *Hin*dIII and subjected to electrophoresis in 3% NuSieve agarose. The PCR product bands were excised from the gel and melted at 68°C for 10 minutes befure ligation to dephosphorylated *Hin*dIII cut pUC 18 (Pharmacia). A control ligation with vector only was also set up. An equal volume of each ligation was used to transform competent DH5 *E. coli*.

The data of Table 1 indicate the cloning efficiency of the various ligations. By colony hybridisation >90% of the proteinase K resulting colonies lit up which is reflected in the frequency of inserts observed in plasmid preparations (>95%). Only 1 positive colony was identified in the 860 non-proteinase K colonies screened by hybridisation, and only this colony displayed an insert in plasmid preparations.

We have used proteinase K treatment of PCR products generated using primers containing various terminal restriction endonuclease sites prior to cutting with the corresponding enzymes and have routinely found a significant increase in cloning efficiency.

REFERENCE

1. Shuldiner,A.R., Scott,L.A. and Roth,J. (1990) *Nucl. Acids Res.* **18**, 1920.

TABLE 1

	Number of colonies	Colony blot positives	Inserts in plasmid preps.
Control ligation	125	N.D.	N.D.
Non-proteinase K	860	1	1/16
Proteinase K	>3000	>90%	19/20

N.D. = Not done