

Membrane bound PCR

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The polymerase chain reaction (PCR) has become a powerful tool in molecular biology in the years since it was invented and many applications are now available (1). We have used a membrane bound template DNA for PCR and obtained results similar to those in a solution PCR. The autoradiogram shows the typical ladders we observe for the repetitive spider silk protein on a gel (2) (Fig. 1). The center two lanes show (on the left) the membrane bound DNA template and the same template in solution (on the right). No substantial differences can be seen. For the membrane bound template, PCR requires 10 to 20 extra cycles, probably due to a lower efficiency of amplification with the bound DNA. This technique would be useful not only for limited amounts of template DNA but also for keeping the original cDNA or genomic DNA in its denatured state. Furthermore in the case of contaminants in the DNA preparation, this method could be used to purify the sample by allowing the membrane bound DNA to be washed extensively before the PCR. DNA may be purified by electrophoresis and then blotted onto the membrane if the PCR shows a high background of side products.

REFERENCES

1. Saiki,R.K. *et al.* (1985) *Science* **230**, 1350-1354.
2. Kadokami,Y. and Lewis,R.V. (1990) in preparation.

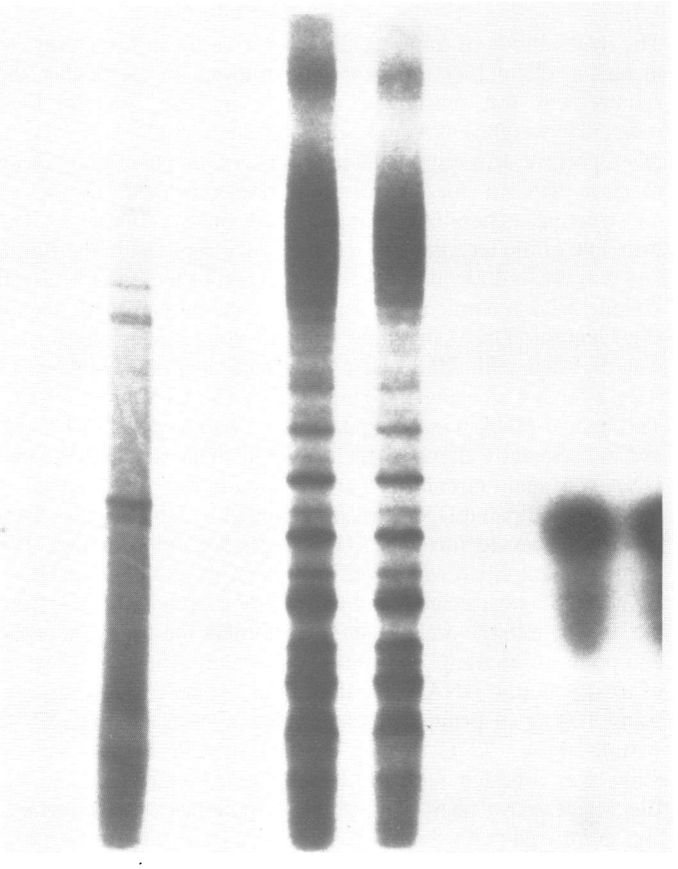

Figure 1. An anchored cDNA (2 μg/5 μl) constructed from a spider abdomen (*Nephila clavipes*) with a BamHI-SmaI adaptor (NEN) was denatured with 5 μl of 0.4N NaOH at room temperature for 30 min and dotted several times on a piece of nylon membrane (Zetaprobe, 3mm×3mm). After completely drying in air, the membrane was neutralized by immersion in 10 ml of 1M Tris-HCl (pH 7.6) for 1 min. Then it was transferred to a beaker which contained 100 ml of dH_2O and rinsed for 1 hour with several changes. The membrane was dried with a piece of Whatman 3MM, and put into a 500μl Eppendorf tube. PCR was done in a 100μl reaction mix (67 mM Tris-HCl (pH 8.8)-16 mM $(NH_4)_2SO_4$-6.7 mM $MgCl_2$-10 mM β-mercaptoethanol-6.7 μM EDTA-0.001% gelatin-0.2 mM dNTPs-100 pmol *Bam*HI-*Sma*I primer (GATCCCCGGG)-100 pmol KadoIIA (GCNGTNGANA PuTTT)-Ampli Taq (2.5 U, Perkin Elmer Cetus) at 92°C for 40 sec for denaturation, 37°C for 50 sec for annealing, and 72°C for 1 min for extension for a total of 50 cycles. 10 μl of each sample was electrophoresed on a 5% sequencing gel, and autoradiographed. A control reaction with the template in solution was amplified as above for 40 cycles.

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