Rapid production of vector-free biotinylated probes using the polymerase chain reaction

Y-M.D.Lo, W.Z.Mehal and K.A.Fleming

University of Oxford, Nuffield Department of Pathology, John Radcliffe Hospital, Oxford, OX3 9DU, UK
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The production of vector-free inserts for use as probes is time-consuming and inefficient. We have developed a method for producing biotinylated vector-free probes using the polymerase chain reaction (PCR).

Method: PCR was carried out as described (1), except that dTTP was 150µM and biotinylated dUTP (BRL bio-11-dUTP) was added to 50µM. The target was 1ng of a plasmid containing a full length hepatitis B virus insert (pHBV130) (2). The 5' and 3' primers (from British Biotechnology Ltd.) were GAGTGTGGATTTCGCACTCCTC and GATTGAGATCTTCTGCGACGC, respectively and were obtained from published sequences (3). Twenty-five cycles (2 minutes at 94°C and 55°C, 3 minutes at 72°C) were carried out manually. After ethanol precipitation, the sample was dissolved in 100µl TE.

Results: The biotinylated amplified product migrated slightly slower than the non-biotinylated PCR product (Fig.1). Size fractionation and confirmation of biotin labelling were done using methods previously described (4). The labelled product formed a single band at the expected size (Fig. 1). When the labelled PCR product was used as a probe in Southern hybridization (4), 1pg of target DNA could be detected (Fig. 2). We investigated alternative methods: after 25 cycles of conventional amplification (without bio-dUTP) the PCR mixture was diluted 10-fold, denatured and bio-dUTP added from 20µM to 50µM. Labelling was then performed at 24°C, 37°C, 55°C or 72°C with or without additional *Taq* polymerase. However, the above protocol was faster, more efficient and economical in use of bio-dUTP and polymerase.

Comments: (1) To avoid non-specific labelling the amount of plasmid DNA should be kept to 1ng or below. With this precaution the method is very specific and as demonstrated in Fig. 3, there is negligible hybridization to the vector band. We estimate that 5-10µg of labelled vector-free probe can be synthesized in less than 4 hours, which is sufficient for many experiments. (2) The method has general application if primers flanking the vector cloning site are used and the target sequence is below 2.5 kb (1). (3) A probe for detection of PCR product amplified from genomic DNA can be produced using a pair of primers internal to that for the genomic amplification. This allows unequivocal, highly sensitive, non-isotopic detection of specific bands amongst many 'ghost' bands.

Figure 1

Figure 2

Fig. 1: Tracks 1-3 4% NuSieve Gel. 1. *Msp*I digested pBR322 (marker). 2. Non-biotinylated PCR product. 3. Biotinylated PCR product. 4. Southern transfer of biotinylated PCR product (from an alkaline denaturing gel).

Fig. 2: Titration of pHBV130 cleaved with *Xho*I to release insert. v=vector, i=insert. 1. 50ng (1ng). 2. 5ng (100pg). 3. 500pg (10pg). 4. 50pg (1pg). 5. 5pg (0.1pg). Figures in brackets are the amounts of target sequences.

References: (1) Saiki, R.K., et al. (1988) Science 239: 487-491. (2) Gough, N.M. and Murray, K. (1982) J. Mol. Biol. 162: 43-67. (3) Ono, Y., et al. (1983) Nucleic Acids Res. 11: 1747-1757. (4) Chan, V.T.-W., et al. (1985) Nucleic Acids Res. 13: 8083-8091.