A simple method for generating single-stranded DNA probes labeled to high activities

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Submitted August 31, 1990

The random priming DNA-labeling method of Feinberg and Vogelstein (1, 2) produces probes of high activities. However, incompletely denatured templates in the reaction mixture may cause problems. In addition, probes generated by the standard random priming method are not ideal for *in situ* hybridization or other methods requiring only one labeled strand. We have developed a method utilizing a biotinylated single-stranded template bound to magnetic microspheres in the standard random priming reaction. The template is generated by a PCR-reaction with one of the two primers biotinylated (Fig. 1). The biotinylated PCR-product is then bound to streptavidin-coated magnetic beads (Dynabeads M-280 Streptavidin, Dynal). The non-biotinylated strand is removed using alkaline treatment and magnetic separation (3). Labeling of the 'purified' biotinylated strand can then be carried out as a one-tube reaction since unincorporated nucleotides are removed by fixing the beads with the magnet and discarding the supernatant. The choice of biotinylated primer decides which strand is to be labeled. In contrast to probes generated by the M-13 system this method can use any vector that can serve as a PCR-template. Compared to RNA-probes the single-stranded DNA probes have the advantage that they are easier to handle; there is no need for enzymatic degradation of the template and contamination by RNase is no problem.

The southern blot in Fig. 2, hybridized with a probe generated by this protocol (a) and with a conventional double-stranded probe (b), shows that the method generates a probe which specifically recognizes only one of the two complementary strands. The single-stranded probes described in this protocol give exposure times of 15–30 hours for genomic blots (8–10 μg DNA per lane) for detection of single copy genes in barley DNA (data not shown).

Protocol:

PCR: 1 ng linearized DNA (450 bp insert of B19, a barley seed cDNA, in the vector pBluescript SK) in 1×PCR-buffer (Promega), 200 mM dNTPs, 1 μM each of T7 and SK primer (one of the two biotinylated), 2.5 U Taq polymerase (Promega), 30 cycles of 1 min/94°C, 2 min/45°C, 3 min/72°C (Temperature cycler; Techne PHC-2).

The biotinylated PCR product (200–300 ng) was bound to 25 μl of magnetic streptavidin beads by incubation in 6×SSC for 2 min. Beads were washed once in 6×SSC and the non-biotinylated strand was removed by two cycles of denaturation in 100 μl of 0.125 M NaOH in 0.1 M NaCl. After washing twice with 1×SSC, 0.1% SDS and twice with H_2O the template was labeled by a standard random priming reaction (Random Primed DNA-labeling Kit, Boehringer Mannheim). The beads were then washed (1×SSC), and the DNA was denatured by heating at 95°C for 5 min. The supernatant (with the radioactive strand) was recovered and used in the hybridizations. Approximately 70% of the radioactivity was measured in the bead fraction after labeling and this corresponds well to the incorporation data given in the kit. 80% of this radioactivity was then eluted using the above denaturing method.

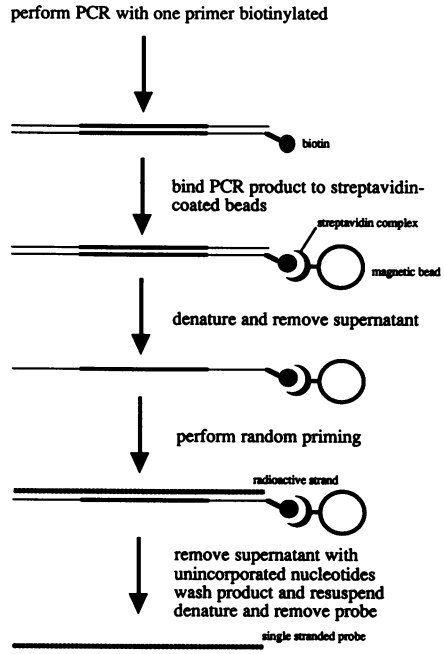

Figure 1. The scheme for producing single-stranded DNA-probes by random priming using magnetic beads.

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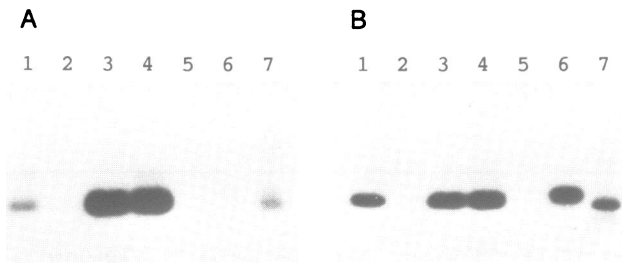

Figure 2. Southern blot hybridized to (a) single-stranded probe derived from a B19 cDNA PCR-product primed with SK and biotinylated T7 (T7-B) and (b) conventional double-stranded B19 cDNA probe. Lanes 1–3 contain the B19 cDNA PCR-product primed with SK/T7-B: 100 ng SK-strand obtained by alkaline denaturing (1), wash (2), 100 ng T7-B-strand recovered after boiling of the beads (3). Lanes 4–6 contain the B19 PCR-product biotinylated on the opposite strand (T7/SK-B): 100 ng T7-strand obtained by alkaline denaturing (4), wash (5), 100 ng SK-B-strand recovered by boiling (6). Lane 7 contains 25 ng of double-stranded B19 PCR-product primed with SK/T7-B.

ACKNOWLEDGEMENTS

We wish to thank Erik Hornes, Apothekernes Laboratorium, Oslo, Norway for the gift of oligonucleotide primers. This work was supported by a grant to K.S.J. from the Norwegian Research Council for Science and the Humanities (NAVF).

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