An efficient method to generate phosphatase insensitive 3′ labelled DNA probes using Taq polymerase

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Band shift analysis has become one of the most frequently used methods for the analysis of DNA/protein interactions. This method requires radioactive labelling of DNA fragments, which is normally done by filling in 5′ overhangs by DNA polymerase or by kinasing 5′ overhangs or blunt ends. These T4 polynucleotide kinase reactions have the disadvantage that 5′ phosphorylated DNA fragments are highly sensitive to endogenous phosphatases present in the crude or partially purified protein extracts that are used in DNA/protein binding reactions. We have used a new technique to label blunt end DNA fragments using Taq polymerase. Apart from its polymerase activity this polymerase also possesses a template independent terminal transferase activity that adds a single nucleotide to the 3′ ends of blunt end DNA molecules (1). The Taq polymerase activity is employed in PCR and sequencing reactions, while the terminal transferase activity is used for the preparation of T-tailed vectors in the direct cloning of PCR products (2).

To prepare DNA probes used in band shift assays we routinely use the template independent activity of the Taq polymerase to label blunt end DNA fragments and the polymerase activity of the enzyme to label 5′ overhangs by fill-in. Where appropriate fill-in and blunt end labelling can be done simultaneously. To label approximately 50 ng (500 fmole) DNA (having blunt ends or 5′ overhanging ends) 2.5 units Taq polymerase (Promega) were added to the DNA and incubated using standard buffers (50 mM KCl, 10 mM Tris pH 8.8, 1.5 mM $MgCl_2$, 1% Triton X100 and 200 μg/ml BSA) in the presence of 10 μCi dNTP (3000 Ci/mmole) in a total of 20 μl. These were covered with 75 μl mineral oil layer and incubated for 2 hours at 70°C. In the fill-in reaction the first nucleotide filled in was radioactively labelled. As described previously Taq polymerase adds preferentially dATP to blunt ends (1). We found that for labelling blunt end DNA dGTP could also be used successfully, while labelling with dCTP was poor. After the labelling reaction the unincorporated nucleotides were separated from the labelled probe using a G50 column. A typical example of this technique is the permutation analysis (3) of the yeast Cpf1 protein bound to labelled CDEI/CEN6 DNA fragments (Figure 1) (4, 5). The DNA fragments were generated by specific restriction enzyme digestion of plasmid pBend2/CDEI/CEN6 (5, 6). The DNA fragments were labelled using Taq polymerase and incubated with crude protein extracts from *Saccharomyces cerevisiae* or *Escherichia coli* (5). The permutation analysis is shown in Figure 1. Labelling blunt end DNA probes by Taq polymerase is an efficient and rapid method for DNA probes that need to be incubated with crude protein extracts for subsequent band shift assays and footprint analysis.

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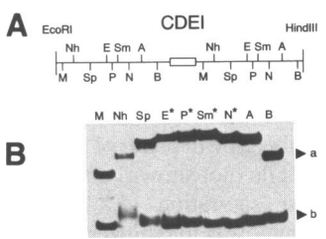

Figure 1. A Schematic representation of the pBend2 polylinker carrying the Cpf1 binding site CDEI (open box) (5). **B** Permutation analysis of CEN6-CDEI DNA/Cpf1 protein complex to detect a protein induced DNA bend. Bent DNA/protein complexes show a slower than expected relative electrophoretic mobility in nondenaturing acrylamide gels. Migration is maximally reduced when the bend is located at the centre of the DNA fragment. DNA fragments of identical length (153 bp) but carrying CDEI at different positions relative to the ends of the DNA fragments were generated using various restriction enzymes (M = MluI, Nh = NheI, Sp = SpeI, E = EcoRV, P = PvuII, Sm = SmaI, N = NruI, A = Acc65I and B = BamHI). All DNA probes were labelled using Taq polymerase (blunt end labelled DNA fragments are marked by an asterisk). The endlabelled DNA fragments (5 fmole/sample) were incubated with 1 μl (10 μg) of crude protein extract from *E.coli* expressing a truncated version of Cpf1 protein (Cpf1b, aa 209– 351 aa) (4). DNA/protein complexes are indicated by (a), the free DNA probes by (b).

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