Hybridization probe size control: optimized 'oligolabelling'

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DNA hybridization probes can be radiolabelled internally with ^{32}P either by nick-translation (1,2) or by oligonucleotide-priming (oligolabelling, 3-4). The latter method has the advantage that two sources of nuclease activity (DNase I and 5'-3' exonuclease) are eliminated, allowing greater control over the size of the end product.

In a further refinement of the oligolabelling method, we have recently discovered that the average size of nascent, single-strand probe DNA is an inverse function of primer concentration: $\text{length} = k/\ln P_0$ (where P_0 is the primer concentration). In addition, we optimized the oligolabelling method with respect to pH, enzyme, temperature, DNA, primer, and buffer components. The resulting formulation presented here enables 70-90% incorporation (after a 1 hour reaction) of label into probe with output specific activity as high as 5×10^7 DPM/ μg .

Figure 1 shows the effect of primer concentration on single-strand size using intact PBR322 plasmid DNA template. Since high specific activity DNA probes labelled with ^{32}P decrease in size rapidly due to radiochemical degradation (5), it is important to control and optimize the size of output DNA. Primer concentration was decreased to as little as 1.7 $\mu\text{g}/\text{ml}$ without adverse effects on efficiency. The inverse relationship between primer concentration and output DNA size may be due to steric hindrance, or to depletion of labelled precursor.

Oligolabelling reactions were carried out in a 20 μl volume. First, 60ng of dp(N) random primer (Pharmacia) was combined with 200ng of template DNA in 4 μl of TE (TE: 10mM tris, pH7.5, 1mM EDTA). After immersion in a boiling water bath for 2 minutes, the mixture was placed on ice. 2 μl of 10X oligolabelling buffer (10X buffer: 0.5M tris, pH 6.9, 0.1M magnesium sulfate, 1mM dithiothreitol, and 0.6mM each of dATP, dGTP, and TTP) was added, as well as 10-140 μCi of ^{32}P -dCTP, water, and Klenow enzyme (Boehringer-Mannheim, 2-10U, total) to volume.

After 60 minutes at 22 $^{\circ}\text{C}$, the reaction was terminated by the addition of 80 μl of stop solution [0.1% SDS in 10mM tris (pH7.5), 1mM EDTA] and 2 μl of 10mg/ml yeast RNA, and the mixture was extracted once with phenol and added to a 2 ml Sephadex G50-80 (Sigma) column equilibrated in 4X SSC (1X SSC is 0.15M sodium chloride, 0.015M sodium citrate, pH 7.0) in a Pasteur pipette. The void volume fractions were pooled, and the probe was immersed in a boiling water bath for 5-10 minutes prior to hybridization.

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References: 1) Kelly, RG, Cozzarelli, N, Deutscher, MP, Lehman, IR, and Kornberg, A. (1970) J. Biol. Chem. 245,39-45. 2) Maniatis, T, Jeffrey, TA, Kleid, DG, (1975) Proc. Nat. Acad. Sci. (USA) 72,1184-1188. 3) Taylor, JM, Illmensee, R, Summers, J. (1976) Biochim. Biophys. Acta 442,324-330. 4) Feinberg, AP, and Vogelstein, B. (1984) Anal. Biochem. 137,266-267. 5) Stent, GS, Fuerst, CR, (1960) Advanc. Biol. Med. Phys. 7,2-71.