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A method for labeling DNA in vitro using nicked translation.

The method used to label N crassa DNA in vitro for hybridization reactions is a modification of the technique used by Maniatis et al. (1975, P.N.A.S. 72:1184-1188) to label lambda DNA. Using the ability of E. coli DNA polymerase I to translate nicks, we artifically nicked DNA with DNAse I and used polymerase I and ^{3}H-dTTP to label DNA, with this technique we hove labelled Neurospora DNA to 3.3 x 10^7 cpm/µg, which represents 26% incorporation of label into DNA. Higher specific activities may be obtained by using more label and other labelled baser. The size of the labelled DNA is 247 bases. DNA up to 400 borer long may be made this way.

The reaction buffer contains 50 mM Tris-HCl, pH 7.8, 1 µg DNA, 5 mM MgCl 2, 10 mM β-mercaptoethanol, 50 µg/ml BSA, 5 µM cold dNTP's, 2.5 µM dTTP containing .750 mCi ^{3}H-dTTP. The reaction mixture is incubated at 15° C for 10 minutes and the reaction is started by adding 1 x 10^{-7} mg DNAse 1. The mixture is incubated one minute with the nuclease when 12.5 units of DNA polymerase I is added. Sampler ore taken over several hours to monitor the % incorporation. The reaction reacher a plateau after about two hours, but incorporation continues up to four hours.

The reaction is stopped by adding 3 ml of .03 M phosphate buffer, .135 M NaCl and .1% SDS, then boiling for 10 minutes. At this point the reaction mixture contains unincorporated ^{3}H-dTTP, labelled DNA and foldback DNA, The foldback DNA is a result of the polymerase displacing DNA strands (instead of hydrolyzing them) and then using the displaced strand as a template. The foldback DNA and unincorporated material may both be removed using hydroxyapatite. The boiled reaction mix is poured on a column of HAP at 50° C. At 50° C only single stranded and double stranded DNA will bind in .03 M phosphate buffer. Extensive washing with .03 M PB will elute the unincorporated material and small fragments. Since foldback DNA contains short double stranded regions it may be separated from the labelled DNA which is single stranded (100° C, 5 min) by eluting the later from the column with .14 M PB. The foldback DNA may be eluted from the column using .5 M PB.

The labelled DNA should then be sired on alkaline sucrose gradients. The DNA we hove labelled and isolated in this manner contains very little foldback DNA and represents 75% of the DNA lobelled. (Supported by Grant GM-23367 from the National Institutes of Health) - - - Department of Biochemistry and Developmental Biology Program, Ohio State Univ., Columbus, Ohio 43210.