Random Primed Labeling of DNA

Random primed labeling is the most efficient and reliable method for labeling DNA. Specific activities of greater than 10^9 dpm/μg DNA are routinely obtained by following the procedure developed by Feinberg and Vogelstein (1,2). Incorporation of label into newly synthesized DNA remains efficient even when the reaction is initiated by less than 20 ng of template DNA.

A labeling reaction is begun by adding denatured DNA to a mixture of random sequence hexameric deoxynucleotides, deoxynucleotide triphosphates (dNTPs), and the Klenow fragment of DNA polymerase I. The oligonucleotides hybridize to complementary sequences on the template DNA and serve as primers for the synthesis of complementary DNA strands by the Klenow enzyme. Greater than 80% of a labeled dNTP can be incorporated into the newly synthesized DNA (1). Probes labeled by this method may be used in Southern, Northern, and *in situ* hybridizations without removal of unincorporated labeled dNTP.

Although the random primed labeling reaction will proceed under a variety of substrate and enzyme concentrations, an optimum ratio of random primers, dNTPs, and Klenow enzyme produces a labeling reaction which quickly reaches a plateau level of label incorporation. 5 Prime → 3 Prime, Inc. supplies pretested kits containing the reagents necessary to optimally label DNA by the random primer method (the researcher supplies the DNA and the labeled dNTP). Unlike the nick-translation method of labeling DNA, the size of the template DNA does not affect the efficiency of random primed labeling as shown by the results of a typical labeling experiment in Table 1.

TABLE 1

DNA	Amount	Size	dpm/μg
linearized pBR322	10 ng	4.4 Kb	1.3 x 10^9
lambda-Hind III fragment	10 ng	2.3 Kb	1.1 x 10^9
lambda-Hind III fragment	10 ng	0.6 Kb	1.3 x 10^9

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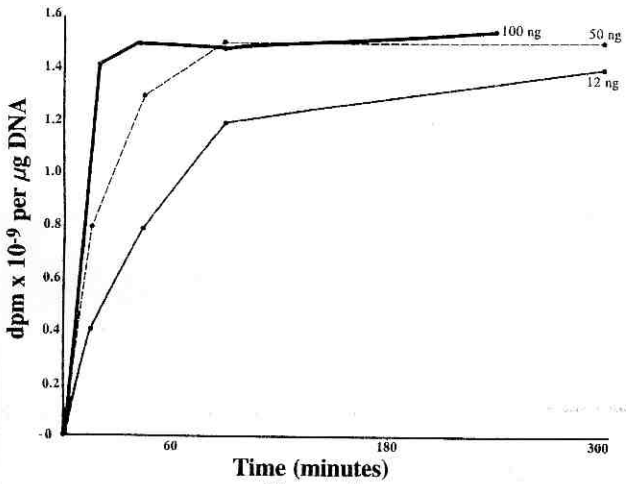

Figure 5

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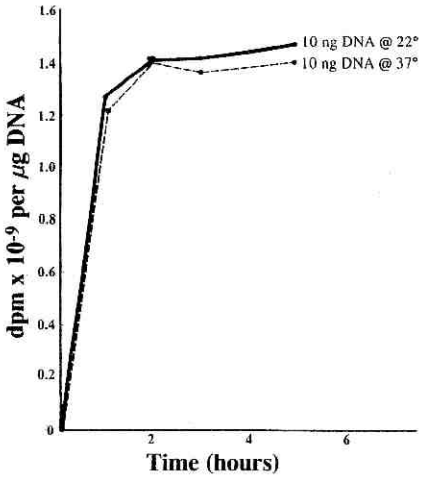

Figure 6

The experimental results shown in Table 1 and Figures 5 and 6 were obtained using a 5 Prime → 3 Prime, Inc. Random Primed Labeling Kit with 50 μCi [alpha ^{32}P] dCTP 3000 Ci/mmole (16 pmoles) and the indicated amounts of DNA. The amount of template DNA added to the reaction will influence the initial rate of label incorporation. However, the final level of label incorporation is similar over a wide range of added template DNA (Figure 5). As shown in Figure 5, the rate of label incorporation increases with increasing amounts of template DNA. Interestingly, the rate of label incorporation is not significantly different whether the reaction is run at 22° or 37° (Figure 6). This results from the concentration of labeled dNTP being the rate limiting factor in the reaction. Thus, for consistent labeling efficiency, regardless of the labeled dNTP chosen, add 16 pmoles of labeled dNTP per reaction. Increasing the number of pmoles of labeled precursor added to the reaction will increase the time required to reach maximal incorporation.

Probe-EZE, 5 Prime → 3 Prime's Random Primed DNA Labeling Kit is provided complete with detailed protocol, Klenow fragment of DNA polymerase I, random primers, control DNA and individual solutions of dATP, dCTP, dGTP and dTTP.

PROBE-EZE

* Catalog Number	Size	Price
5301-787533	15 reactions	60.00
5301-834335	40 reactions	130.00

*Wet Ice Shipment Required

References:

1. Feinberg, A.P., and Vogelstein, B. 1983. Anal. Biochem. **132**:6-13.
2. Feinberg, A.P., and Vogelstein, B. 1984. Anal. Biochem. **137**:266-267.