Optimized Random Primed Labeling:

Influence of template concentration and size

Introduction

DNA probe synthesis using the random-primed method of Feinberg and Vogelstein (1,2) has become an important technique for molecular biologists. When performed properly, the method can produce high specific activity radiolabeled probes with speed (30 minute reaction time) and efficiency (specific activity $> 1.6 \times 10^9$ dpm/μg). We have investigated some of the critical parameters of random primed labeling reactions. In this report, we describe our results regarding the influence of template concentration and template size (length) on probe specific activity.

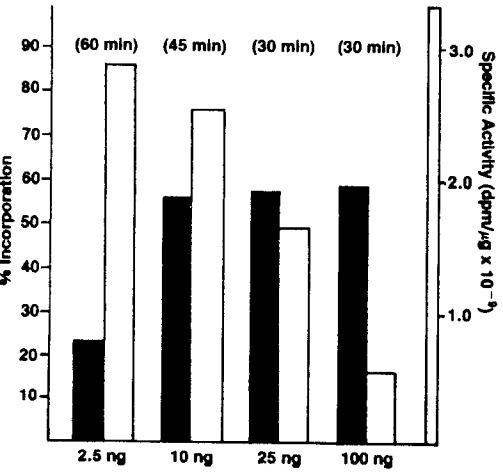

Figure 1. Bar graph showing the effect of labeling different DNA template concentrations. Four different quantities of linear pBR322 (2.5 ng, 10 ng, 25 ng, 100 ng) were denatured by boiling and then labeled in parallel reactions using the BMB Random Primed Labeling kit. Aliquots of the reactions were removed at 15 minute time intervals. The data presented is from a time point showing peak incorporation; the time is indicated above the bars in parentheses ( ). Percent incorporation *(solid bars)* was determined by dividing TCA-precipitable counts by total counts. Specific activities *(open bars)* were calculated by dividing the amount of incorporated radioactivity by the total amount of DNA present at the end of the reaction (starting amount plus newly synthesized). The incorporated radioactivity (dpm) was calculated by multiplying the percent incorporation by the number of μCi added to the reaction (50 μCi), and then multiplying by 2.2×10^6 dpm/μCi. The amount of newly synthesized DNA was calculated by multiplying the percent incorporation by the number of picomoles of labeled nucleotide added, multiplying by 4, and then dividing by 3 (3 picomoles dNTP = ng).

Template concentration

An important characteristic of the random primed labeling method is that only small amounts of DNA template are required to achieve high specific activity probes. Figure 1 shows the results of an experiment in which four different quantities of template were labeled using the BMB Random Primed Labeling Kit. Both percent incorporation and specific activity were determined. Note that, for the smaller quantities of DNA (2.5 ng, 10 ng), specific activities of greater than 2.5×10^9 dpm/μg were achieved. Percent incorporation is low because the limiting component under these conditions is the template concentration. Essentially, both strands of the template DNA have been primed and duplicated in these reactions, and a large proportion of the isotope remains unutilized.

The situation changes when higher DNA concentrations are labeled. Figure 1 shows that for template amounts ≥ 25 ng, the specific activity of the resulting probes is lower. This occurs because, under conditions of excess template, the limiting reaction component becomes the isotopically-labeled nucleotide. In this situation, the majority of the isotope becomes incorporated into labeled probe. Further incorporation is hindered because the remaining isotope concentration is so low that enzymatic utilization is kinetically difficult. Including excessive amounts of template DNA in the labeling reaction does nothing to increase the percent incorporation, and only serves to dilute the final specific activity of the probe.

Our experiments also showed that the reaction kinetics change when different template concentrations are used. The lower concentrations (2.5 ng, 10 ng) required 45–60 minutes to reach peak incorporation while the higher template concentrations were complete after approximately 30 minutes.

Template size

It is desirable to have a method capable of labeling DNA fragments of a variety of sizes. It is known that the nick translation labeling procedure first described by Rigby *et al.* (3) is inefficient for labeling smaller DNA fragments. In the experiment

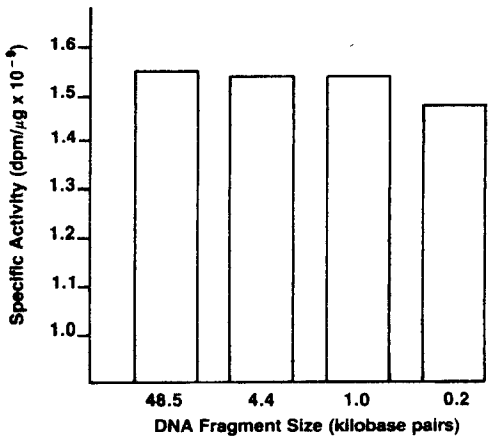

Figure 2. Bar graph showing the effect of labeling different sized DNA templates. Four different template sizes were used (48.5 kb lambda DNA, 4.4 kb linear pBR322 DNA, 1.0 kb pBR322 restriction fragment, 200 bp pBR322 restriction fragment). 25 ng of each template was labeled to peak specific activity (30 min.) in parallel reactions. The specific activity was calculated as described in Figure 1 above. Note that all the fragments were labeled with nearly the same efficiency.

shown in Figure 2, we assayed the ability of the random primed labeling method to label templates ranging in size from 48,500 bp to 200 bp. The results showed very little difference in the final specific activity of the probes. The ability of the random primed method to efficiently label DNA's of a variety of sizes is a major advantage of the technique.

Conclusions

Our experiments indicate that random primed labeling reactions containing small quantities (≤ 25 ng) of DNA template produce the highest specific activity probes. If limiting template concentrations are used, "hot" probes can be produced even though percent incorporation values are low. Using small quantities of template necessitates that slightly longer incubation times be employed. Template size does not significantly influence the efficiency of random primed labeling reactions.

Product	Cat. No.	Size	Price
Random Primed Labeling Kit	1004 760	50 reactions	$163.00

References

1. Feinberg, A. and Vogelstein, B. (1983) *Anal. Biochem.* **132**:6
2. Feinberg, A. and Vogelstein, B. (1984) *Anal. Biochem.* **137**:266
3. Rigby, P. *et al.* (1977) *J. Mol. Biol.* **113**:237

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