

Oligonucleotide 5' End Labeling

1. Introduction

The techniques for end labeling oligonucleotides with radioisotopes have driven nucleic acid probe technology. Oligonucleotide probes can be custom made based on sequence information of the target DNA or RNA in several hours on a DNA synthesizer. Use of a DNA synthesizer eliminates the usual cumbersome and time consuming steps involved in cloning and isolation of restriction fragments to be used as probes. Oligonucleotide probes are highly specific and can be designed to detect single base changes in a gene.

Synthetic oligonucleotides prepared with free 5' and 3' hydroxyl groups will not require pretreatment with bacterial alkaline or calf intestinal phosphatase. End labelling of oligos is also much simpler than double stranded DNA molecules because there is no need to modify reaction conditions depending upon whether the fragment has 5' or 3' overhangs or blunt ends, as with restriction fragments. Oligos can be labeled at either the 3' or the 5' end. Using polynucleotide kinase and ATP (γ - ^{32}P), the 5' end is labeled. Using terminal transferase and deoxynucleotide triphosphate the 3' end is labeled. ^{32}P or ^{35}S nucleotides can be used for labeling. Oligos can also be labeled on the 3' end using 5-iododeoxycytidine 5'-triphosphate (^{125}I). Use of ^{35}S and ^{125}I labeled oligos are especially useful when high resolution (as in *in situ* hybridization) or when long probe stability is needed.

2. Principle

Polynucleotide kinase, simultaneously identified by Richardson and Novagrodsky and Hurwitz in T-phage infected *E. coli*, catalyzes the transfer of the gamma phosphate of ATP to the 5' hydroxyl terminus of DNA or oligonucleotide molecules. Oligonucleotides can be labeled with radioactivity if the gamma phosphate of ATP contains phosphorus-32 or phosphorus-33. Labeling the 5' ends with ^{35}S utilizing ATP (gamma thiophosphate, ^{35}S) is not recommended due to the slow rate of the enzyme with ATP α S as substrate. Complete phosphorylation of the 5' hydroxyls is achieved using low concentrations of ATP, usually a two-to-five fold excess over the concentration of 5' hydroxyl ends.

3. Explanation

This protocol is an adaptation of a procedure described by Maniatis.

4. Materials needed

- ^{32}P or ^{33}P ATP labeled on the gamma phosphate
- T4 polynucleotide kinase (NEE101)
- Reaction buffer
- Oligonucleotide
- Purified water

5. 5' end labeling reaction

1. Preparation of oligo for end labelling: prior to the end labelling, it is recommended that the oligonucleotide be purified to remove any contaminating salts, reagents, organic solvents, or protein which might affect the reaction.
2. Add 25 μL total of oligonucleotide + reaction buffer (10 pmoles of oligo) on ice in a tube. Mix and briefly centrifuge components to bottom of tube.
3. Add deionized water so that the final reaction volume will be 50 μL .
4. Add tracer to the reaction tube. The number of picomoles of radiolabeled ATP (labeled on the gamma phosphate) should be at least 2.0 times the number of picomoles of 5' ends. For labelling 10 pmoles of oligo, 20 pmoles of radiolabeled ATP is needed (approximately 10 μL of BLU502Z). Mix the contents well and centrifuge briefly.
5. Add 3 μL (3 units) of polynucleotide kinase. Mix the reaction and incubate at recommended temperature for recommended length of time, according to enzyme supplier.
6. Stop reactions by adding 5 μL of 0.25 M EDTA. Keep on ice.
7. Purify by method of choice.

Reaction summary:

<u>Reagent</u>	<u>Volume (μL)</u>
Oligonucleotide (10 pmol)	5-25 μL
10X reaction buffer	5 μL
Radiolabeled ATP	10 μL
Water	7-27 μL
T4 PNK	3 μL

1 O.D. = 20 $\mu\text{g}/\text{mL}$; average molecular weight dNMP: 325

6. References

1. Novogrodsky, A. & Hurwitz, J. The enzymatic phosphorylation of ribonucleic acid and deoxyribonucleic acid. I. Phosphorylation at 5'-hydroxyl termini. *J. Biol. Chem* **241**, 2923-2932 (1966).
2. Richardson, C.C. Phosphorylation of nucleic acid by an enzyme from T4 bacteriophage-infected *Escherichia coli*. *Proc. Natl. Acad. Sci. U.S.A* **54**, 158-165 (1965).