GENProtocols

Protocol

Radiolabelling Nucleic Acids with [γ-32P] ATP for Analytical Assays

ANALYTICAL TESTING

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Abstract

Nucleic acids are readily modified with tags that enable detection, purification, or tracking their interactions with other molecules. One of the standard labels used to generate nucleic acid probes includes radioactive phosphates because they allow for sensitive and rapid detection. This protocol describes the 5′-end labelling of DNA oligonucleotides (<100 nucleotides) with polynucleotide kinase (PNK) and adenosine triphosphate labelled on the gamma phosphate group with 32P ([γ-32P] ATP).

Introduction

The technique of 5′-end labelling oligonucleotides with polynucleotide kinase and [γ-32P] ATP is an ultrasensitive method for detecting nucleic acid sequences and can be used to detect single base changes in a gene. It is widely used in applications ranging from simple shift and pull-down assays to detecting and quantifying carcinogen–DNA adducts (Phillips and Arlt 2007).

The primary step in the 5′-end labelling of a DNA oligonucleotide is an enzymatic reaction catalyzed by polynucleotide kinase. This enzyme was identified by Richardson, Novagrodsky, and Hurwitz in T-phage infected E. coli, and it catalyzes the transfer of the gamma phosphate of ATP to the 5′ hydroxyl terminus of oligonucleotide molecules (Novogrodsky et al. 1966) (Richardson 1965).

Figure (1) shows an illustration of the kinase reaction.

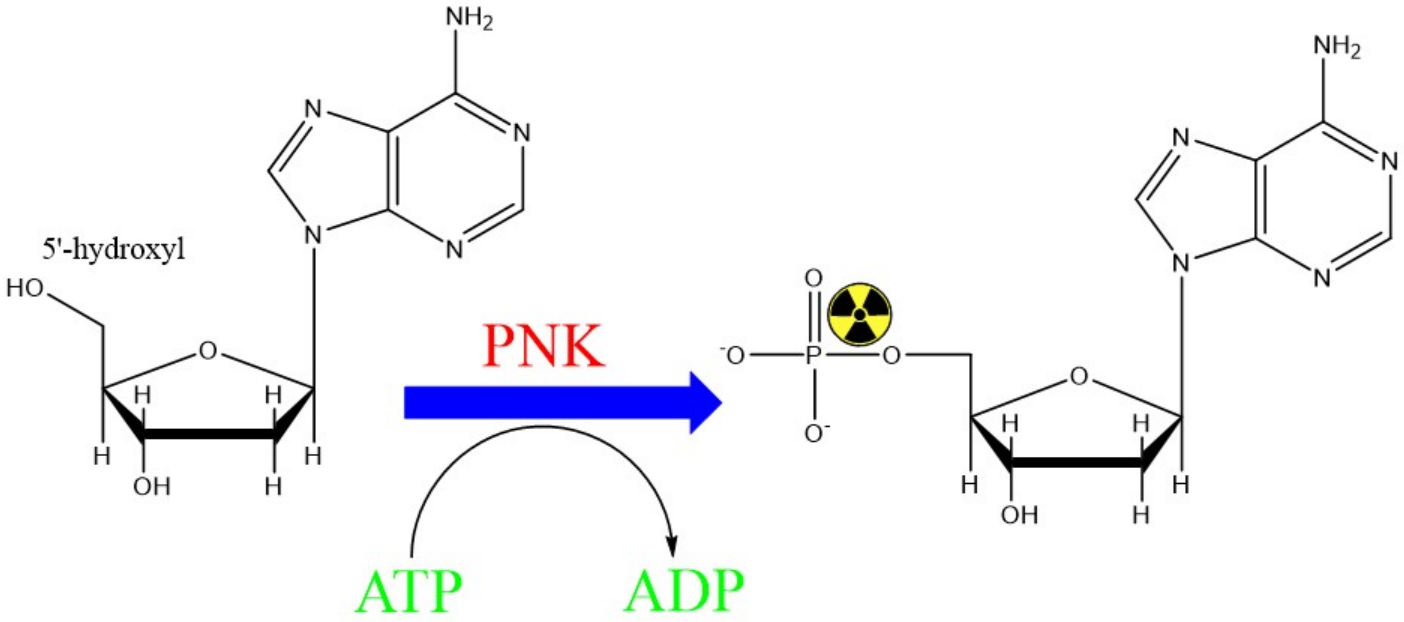

Figure 1: The kinase chemical reaction

Reagents and Equipment

Reagents

1. Synthetic (<100 nucleotides) DNA oligomer prepared with free 5′ hydroxyl groups (see notes and comments).
2. [γ-32P] ATP is from PerkinElmer Life (33 µl, 185 Mbq; 6000 Ci/mmol), catalogue number is NEG035C001MC (see notes for proper handling, safety, and storage).
3. T4 polynucleotide kinase is from New England BioLabs Inc. (10,000 units/ml), catalogue number is M0201L. Store at -20°C and keep the tube in ice when using.
4. T4 Polynucleotide Kinase Reaction Buffer (catalogue number B0201S). This buffer is supplied by New England Biolabs as a 10X reaction buffer. The buffer assures optimal activity of the enzyme at 1X concentration. The components of the 1X reaction buffer are: 70 mM Tris-HCl, 10 mM MgCl2 , 5 mM DTT, pH 7 at 25°C
5. Distilled deionized water (ddH2O)
6. 0.5 M EDTA, pH 8
7. 10% Piperidine

Equipment

1. Geiger–Müller counter (see notes and comments for safety)
2. Plexiglass shielding (see notes and comments for safety)
3. Designated work area and fume hood for working with radiation (see notes and comments for safety)
4. Speed vacuum
5. 37°C water path

Procedure

1. Place the [γ-32P] ATP bottle into the fume hood behind the Plexiglass shield. If frozen, wait for at least 30 minutes for complete thawing.
2. Add 1 µL of DNA oligomer (~ 50 pmol) to 19 µL of 10% piperidine, then mix and incubate at 90 °C for 30 minutes.
3. Speed vacuum to get rid of any residual piperidine. You may want to dilute the Piperidine by adding ddH2O and then speed vacuum.
4. Prepare your reaction mixture (20 µL total) in a polypropylene 0.6 mL micro-centrifuge tube:

1. Add 16 µL of dd H2O to the dry DNA pallet.
2. Add 2 µL of 10X T4 Polynucleotide Kinase Reaction Buffer.
3. Add 1 µL of [γ-32P] ATP. Take caution as the mixture now is radioactive, and every following step must be done behind a plexiglass shield! (see notes and comments for safety).
4. Add 1 µL of T4 polynucleotide kinase.
5. Close the cap of the tube and mix gently.
6. Incubate the reaction mixture at 37 °C for 30 minutes.
7. To stop the reaction, add 2 µL of 0.5 M EDTA, pH 8.
8. Purify the labelled DNA by standard gel electrophoresis purification. This step is highly recommended to get rid of the excess free [γ-32P] ATP.

Time Taken

3 hours

Notes and Comments

1. Synthetic oligonucleotides prepared with free 5′-hydroxyl groups will not require pretreatment with bacterial alkaline or calf intestinal phosphatase.
2. End-labelling of oligos is much simpler than double-stranded DNA molecules because there is no need to modify reaction conditions depending upon whether the fragment has 5′-overhangs or blunt ends, as with restriction fragments. However, end-labelling is not ideal for oligos greater than 100 nucleotides.
3. General safety procedures when working with 32P isotope:
    - High energy beta particles from 32P have a half-life of 14.3 days and may cause damage to the eye and skin if not handled properly, so always wear appropriate PPE (lab coat, goggles, disposable latex or plastic gloves).
    - Before working with radiation, set up a designated working area to minimize the movement of radioactive materials.
    - The setup must include a Geiger–Müller counter (see figure 2), ¼ inch plexiglass shielding and tube racks.

Figure 2: Geiger–Müller counter

- The bench area and fume hood must be clearly delineated with radiation warning tape (see figure 3).

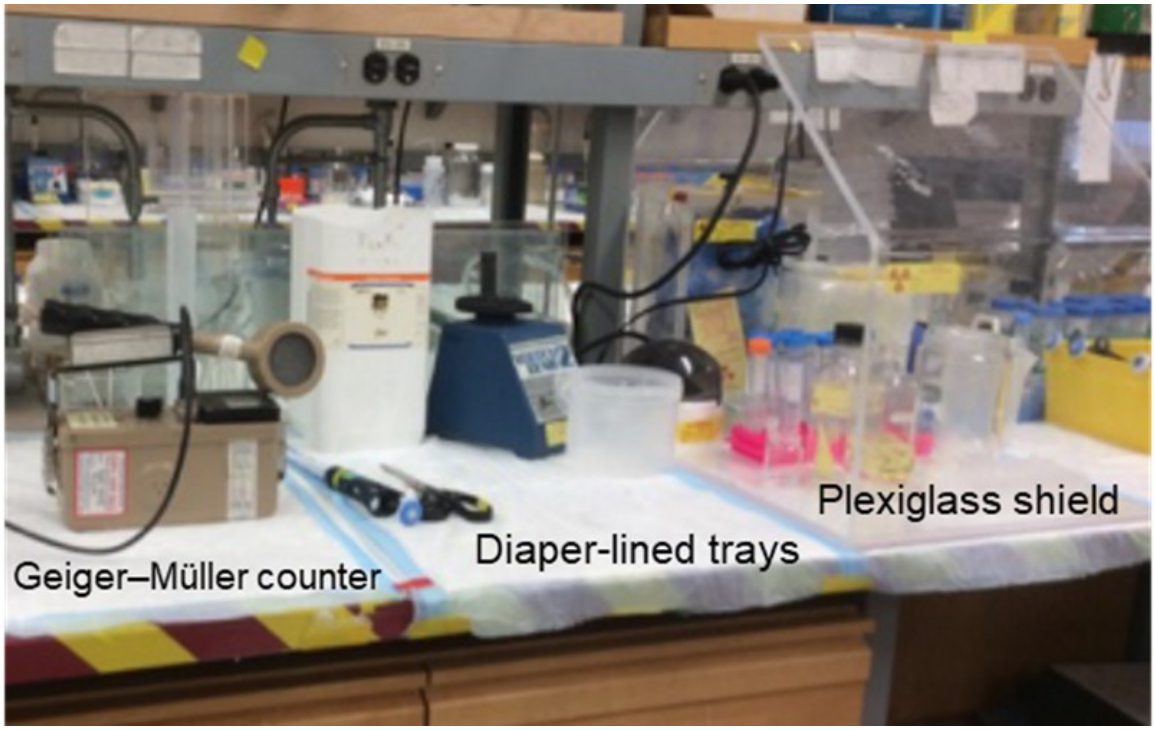

Figure 3: Example of a radioactive working area

- A clear radiation warning sign must be posted with labels indicating that 32P is in use (see figure 4).

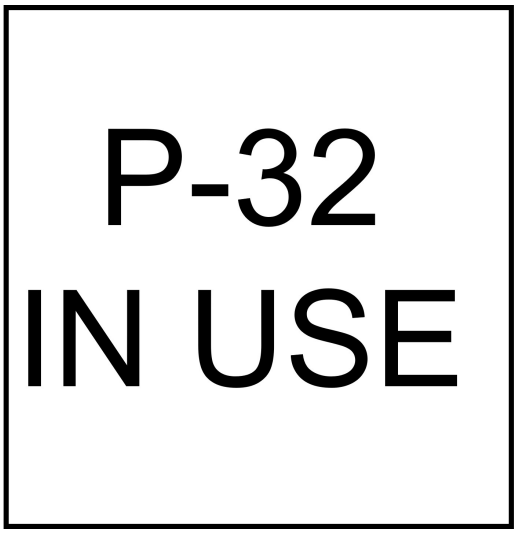

Figure 4: Radiation Warning Sign and example of "in use label"

- Bench cover should be used with the absorbent surface facing up.
- Where large volumes of liquids are handled, the work should be done in diaper-lined trays such that if a spill does occur, all the liquid is contained.
- Chairs or stools are required to be covered with a vinyl or plastic sheet.
- Continuously monitor the work area, gloves and lab coats for potential contamination.
- Users handling stock solutions of 50 MBq or more are requested to wear finger-ring badges to monitor skin dose.
- Use inventory sheet to track radioactive materials
- Check for contamination after finish and perform a swipe test.
- Label each waste container with a waste tag, noting waste type, room of origin, permit. Follow the regulations of your institution for the proper disposal of radioactive materials (see figure 5).

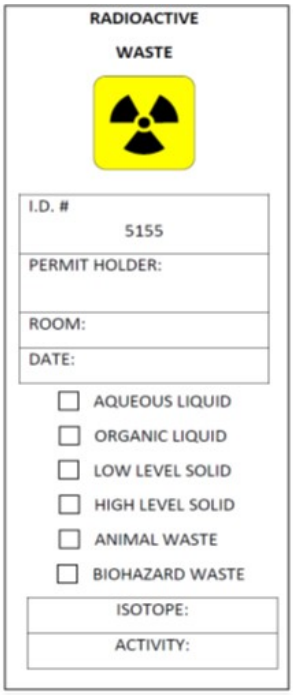

Figure 5: Sample radioactive wasting tag.

References

1. Novogrodsky, Abraham, Moshe Tal, Abraham Traub, and Jerard Hurwitz. 1966. "The Enzymatic Phosphorylation of Ribonucleic Acid and Deoxyribonucleic Acid." *Journal of Biological Chemistry* 241 (12): 2933–43. https://doi.org/10.1016/S0021-9258(18)96554-3.
2. Phillips, David H, and Volker M Arlt. 2007. "The 32P-Postlabeling Assay for DNA Adducts." *Nature Protocols* 2 (11): 2772–81. https://doi.org/10.1038/nprot.2007.394.
3. Richardson, C. C. 1965. "Phosphorylation of Nucleic Acid by an Enzyme from T4 Bacteriophage-Infected Escherichia Coli." *Proceedings of the National Academy of Sciences* 54 (1): 158–65. https://doi.org/10.1073/pnas.54.1.158.

Acknowledgments

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Associated Publications

https://doi.org/10.1016/S0021-9258(18)96554-3.

https://doi.org/10.1073/pnas.54.1.158