3’-O-(2-nitrobenzyl)-2’-dATP Incorporation Detection with PAGE Assisted Precision

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Not Peer Reviewed
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1 Procedure Purpose

Determine if the the modified nucleotide, 3’-O-(2-nitrobenzyl)-2’-dATP, can be noticeably incorporated by Terminal Deoxynucleotidyl Transferase in "standard conditions" while determining the blocking efficacy & purity of our 3’-O-(2-nitrobenzyl)-2’-dATP stock.

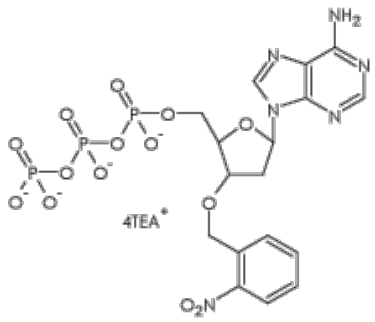

Figure 1: 3’-O-(2-nitrobenzyl)-2’-dATP

2 Overview

This lab will attempt to append 3’-O-(2-nitrobenzyl)-2’-dATP to a **very short (5bp) primer**. The effectiveness of this attempt will be determined by attempting to form a homopolymer on the modified primer. If a homopolymer is formed, the blocking groups did not effectively prevent their formation. This could be due to many reasons (the most likely of which being that the blocking groups either (1) were appended without the 2’ nitrobenzyl due to sample degradation or (2) were not appended). If the homopolymer was not formed (but a homopolymer was formed on the controls) it follows that the blocking groups prevented the formation of the homopolymer, likely due to them preforming their intended function. Moreover, all samples will be run on a PAGE gel in order to achieve single nucleotide resolution. This will allow us to confirm that the 3’-O-(2-nitrobenzyl)-2’-dATP is the only base appended to the "blocked" sample. A ddATP control will help as well.

3 Safety Information

1. **SYBR Gold** has no data available addressing the mutagenicity or toxicity of SYBR® Gold nucleic acid gel stain. Because this reagent binds to nucleic acids, it should be treated as a potential mutagen and handled with appropriate care. The DMSO stock solution should be handled with particular caution as DMSO is known to facilitate the entry of organic molecules into tissues.[?]
2. **Terminal Deoxynucleotidyl Transferase** is toxic if inhaled. May cause cancer. Toxic to aquatic life with long lasting effects. Avoid breathing dust/fume/gas/mist/vapours/spray. Use personal protective equipment as required. If Inhaled: Remove victim to fresh air and keep at rest in a position comfortable for breathing. Dispose of contents/container in accordance with local/regional/national/international regulations.[?]
3. Working in a communal lab space is dangerous. Do not assume your fellow workers cleaned up sufficiently

4 Materials

- Primer: ATGGACATGCCCCTACTTGCATAAG
- 100mM 3'-O-(2-nitrobenzyl)-2'-dATP Stock
- 100mM dNTP Stock
- 100mM dATP Stock
- 100mM ddATP Stock
- 5X Terminal Deoxynucleotidyl Transferase Buffer
- Terminal Deoxynucleotidyl Transferase Stock (20U/µL)
- Nuclease Free Water
- TBE Buffer
- 15% Urea Denaturing Gels
- SYBR Gold

5 Procedure

5.1 Sample Preparation

1. Remove 3'-O-(2-nitrobenzyl)-2'-dATP, Terminal Deoxynucleotidyl Transferase, primer, Terminal Deoxynucleotidyl Transferase buffer, ddATP stock and dATP stock from -20°C freezer
2. Let 3'-O-(2-nitrobenzyl)-2'-dATP thaw on ice in dark
3. Other reagents can thaw on ice in the light

5.2 Attempted blocking

4. Label three PCR Tubes A, B and D, respectively
5. Pipette 10µL of nuclease free water into both PCR Tubes
6. Pipette 4.0µL 5X Terminal Deoxynucleotidyl Transferase reaction buffer into both PCR Tubes
7. Dilute Nucleotides:
   (a) Label a PCR Tube "dATP Dilute"

(b) Pipette 9µL of nuclease free water into PCR Tube
(c) Pipette 1µL of dATP stock into PCR Tube
(d) Vortex directly before use

(a) Label a PCR Tube "dd Dilute"
(b) Pipette 9µL of nuclease free water into PCR Tube
(c) Pipette 1µL of ddATP stock into PCR Tube
(d) Vortex directly before use

NOTE: This step can be skipped, as this dilute has already been made. The dilution instructions are redundant, and are here in case we want to repeat the experiment in the future.

(a) Label a PCR Tube "**BdATP** Dilute" (make this *very* clear)
(b) Pipette 9µL of nuclease free water into PCR Tube
(c) Pipette 1µL of 3'-O-(2-nitrobenzyl)-2'-dATP stock into PCR Tube
(d) Keep in **dark** and vortex directly before use

8. Pipette .5µL of primer into all three PCR Tubes
9. Pipette 2µL of dATP dilute into PCR Tube A
10. Pipette 2µL of 3'-O-(2-nitrobenzyl)-2'-dATP dilute into PCR Tube B
11. Pipette 2µL of ddATP dilute into PCR Tube D
12. Gently pipette 1.5µL Terminal Deoxynucleotidyl Transferase (20 U/µL) into all three PCR Tubes
13. Incubate sample at 37°C for 30 minutes
    Note: **Do NOT** deactivate Terminal Deoxynucleotidyl Transferase

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OPTIONAL STOP POINT

5.3 Extending

Based off of our standard Terminal Deoxynucleotidyl Transferase extending procedure [**?**].

14. Label two PCR Tubes C and X, respectively
15. Pipette 10µL nuclease free water into PCR Tube C (see above, **ATTEMPTED BLOCKING**)
16. Pipette 10µL of nuclease free water into PCR Tube X (see above, **CONTROLS**)
17. Pipette .5µL of primer into both PCR Tubes
18. Set PCR Tube X aside.
19. Pipette 4.0µL 5X Terminal Deoxynucleotidyl Transferase reaction buffer into PCR Tube C
20. Pipette .6µL of dNTP stock into PCR Tube **C**
21. Pipette .4µL of dNTP stock into PCR Tubes **A, B**
22. Gently pipette 1.5µL Terminal Deoxynucleotidyl Transferase (20 U/µL) into **PCR Tube C** (note, this is not from the standard 15UµL stock)
23. Incubate **all** samples at 37°C for 30 minutes

RECOMMENEDED STOP POINT

5.4 Analysis

5.4.1 Prepare Gel

24. Prepare a 15% Denaturing Urea-PAGE gel. Rinse each well with 15 uL of .5X TBE buffer. Allow to pre-run at 200V for 20 minutes prior to continuing.

5.4.2 Run Gel

NOTE: Ensure that the loading dye has Bromophenol Blue in it.

25. Add 8µLof samples A, B, D, C and X with 1.5µL of loading dye for a total volume of 8µLto gel respectively, left to right with the wells at the top.
26. Add 10µL of Loading Dye to the well twice to the right of X (the seventh well from the left).
27. Add an appropriate amount of ladder (annotate the amount added on this sheet) the the far right well (wells at the top).
28. Add a 50/50 combination of sample **B** and sample **C** to the sixth well. That is, add **3µL** of sample **B**, **3µL** of sample **B** and **1.2**µL of loading dye to the sixth well, from the left with the wells at the top.
29. Run at 200V
30. Remove gel once the Bromophenol Blue has almost reached the $\frac{3}{4}$ point [**?**]

5.4.3 Stain & View Gel

31. While the gel runs, prepare 1X SYBR Gold Staining Solution with TBE as dilute
32. Once gel has finished running, ***lightly*** agitate gel while submerged in solution for 40 minutes.
33. Review gel with gel viewer. Until unnecessary, place gel back in stain for 20 minute increments and re-image.
34. Post pictures to Slack.

Stop Procedure

1. Pipette samples into PCR tubes if not already contained in an appropriate manner
2. Label containers if not already labeled
3. Freeze samples at -20°C